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A test of the hierarchical model of litter decomposition

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Our basic understanding of plant litter decomposition informs the assumptions underlying widely applied soil biogeochemical models, including those embedded in Earth system models. Confidence in projected carbon cycle–climate feedbacks therefore depends on accurate knowledge about the controls regulating the rate at which plant biomass is decomposed into products such as CO₂. Here we test underlying assumptions of the dominant conceptual model of litter decomposition. The model posits that a primary control on the rate of decomposition at regional to global scales is climate (temperature and moisture), with the controlling effects of decomposers negligible at such broad spatial scales. Using a regional-scale litter decomposition experiment at six sites spanning from northern Sweden to southern France—and capturing both within and among site variation in putative controls—we find that contrary to predictions from the hierarchical model, decomposer (microbial) biomass strongly regulates decomposition at regional scales. Furthermore, the size of the microbial biomass dictates the absolute change in decomposition rates with changing climate variables. Our findings suggest the need for revision of the hierarchical model, with decomposers acting as both local- and broad-scale controls on litter decomposition rates, necessitating their explicit consideration in global biogeochemical models.

The dominant conceptual model of litter decomposition proposes that the primary controls of the rate of decomposition are climate, litter quality and decomposer organisms¹. These controls are hypothesized to operate hierarchically in space, with climate and litter quality co-dominant at regional to global scales^{2–4}, and decomposers operating only as an additional local control, the effect of which is negligible at broader scales⁵. Therefore, decomposers have been omitted as controls from biogeochemical models. However, a recent surge of interest in their inclusion has shown that carbon-cycle projections depend strongly on whether and how microbial decomposers are represented^{6–9}. Yet evidence that microbial decomposers regulate decomposition rates at regional to global scales, independent of climate variables, such as temperature and moisture, is generally lacking. One possibility for this absence of evidence is suggested by scaling theory, which postulates that the influence of mechanisms that act locally can be obscured by emergent, broad-scale patterns¹⁰.

Pattern and scale have been described as the central issue in ecology, where the inherent challenge to prediction and understanding lies in the elucidation of mechanisms that commonly operate at different scales to those for which the patterns are observed¹⁰. This scale mismatch appears to be true for at least some of the ecosystem processes, such as plant productivity^{10,11}. Decomposition processes are also controlled by variables operating at finer scales than those at which the variables are typically measured and evaluated¹. For example, extensive empirical support for the hierarchical model of litter decomposition has been provided through multi-site climate gradient studies^{12–15}. These multi-site studies have some common characteristics, which include the collection of few observations (typically two to four per site per litter species per collection)—from which a mean decomposition rate is determined—and the use of site-mean data to estimate climatic controls¹. However, the hierarchical model, and its representation in the structure of biogeochemical models, is based on the assumption that controls act at the microsite

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level, by regulating the activities of decomposer organisms^{5,16}. That is, the hierarchical model is conceptually grounded in local (that is, microsite) dynamics, but has been developed and substantiated with site-mean data that represent climate control of decomposition as an among-site relationship.

Understanding controls on litter decomposition across regional scales is therefore necessarily intertwined with scaling theory. This body of theory¹⁰ suggests that broad-scale patterns might emerge from distinct, local-scale causative relationships, and this contrasts with the assumption of the hierarchical model that among-site patterns in decomposition approximate patterns operating at the microsite (Fig. 1). We refer to this as the ‘assumption of scale invariance’ (Fig. 2a). Two lines of evidence question the validity of the assumption of scale invariance for litter decomposition. First, the activities of decomposer communities are shaped by environmental selection for a subset of functional traits that then uniquely dictate how decomposition rates respond to changing climatic controls^{17–20}. Second, microclimate can vary strongly within a site^{21,22}. As such, site-mean climate data are likely to be a poor surrogate for the range in microclimate that is experienced by decomposer organisms within a site²¹. Both lines of evidence support the possibility that among-site patterns in decomposition rates emerge from distinct microsite-level relationships (the ‘assumption of scale dependence’, Fig. 2b).

We used a multi-site, litter decomposition study to test the competing assumptions of scale invariance and dependence (Figs. 1,2). We worked across a climate gradient in Europe at six grassland sites spanning a boreal climate in northern Sweden to a Mediterranean climate in southern France. We predicted that two specific patterns would emerge if the assumption of scale invariance were to be

falsified. Prediction 1 was that relationships between climate and decomposition rates should differ when site-mean versus microsite-level climate data are analysed. That is, the emergent regional-scale pattern from microclimate data should differ from the pattern observed with site-mean climate data. Prediction 2 was that any variable expected to be an important control at the microsite-level (e.g. microbial biomass), should have a strong effect when regional-scale patterns are analysed using microsite-level data.

Litter quality was included in our experimental design, by using two grass species with contrasting litter functional traits, but was not under test. Instead, standardizing known controlling variables can improve estimated effects of other controls that are being studied. In addition, litter traits are expected to interact with controls, such as temperature²³, and so by including this variable, we could test this possibility. In total, we measured four controls (temperature, moisture, microbial biomass and soil nitrogen availability) that naturally varied among microsites. All four variables are expected to act as strong local and, in the case of the climate variables, broad-scale controls on decomposition^{1,5,24,25}. We then built a set of regression models, structured to represent and test between assumptions of scale invariance versus dependence in controls (see Methods), to compare the estimated effect sizes of these different variables on litter decomposition rates.

Results and discussion

Decomposition rates varied within and among sites and between the two litter types (Fig. 3a,b). As expected, mass carbon (C) loss over the three-month field incubations was approximately twice as high for the higher quality *Holcus* litter ($33.8 \pm 11.62\%$; mean \pm s.d.) than for the *Festuca* litter ($16.8 \pm 7.15\%$). However, there was

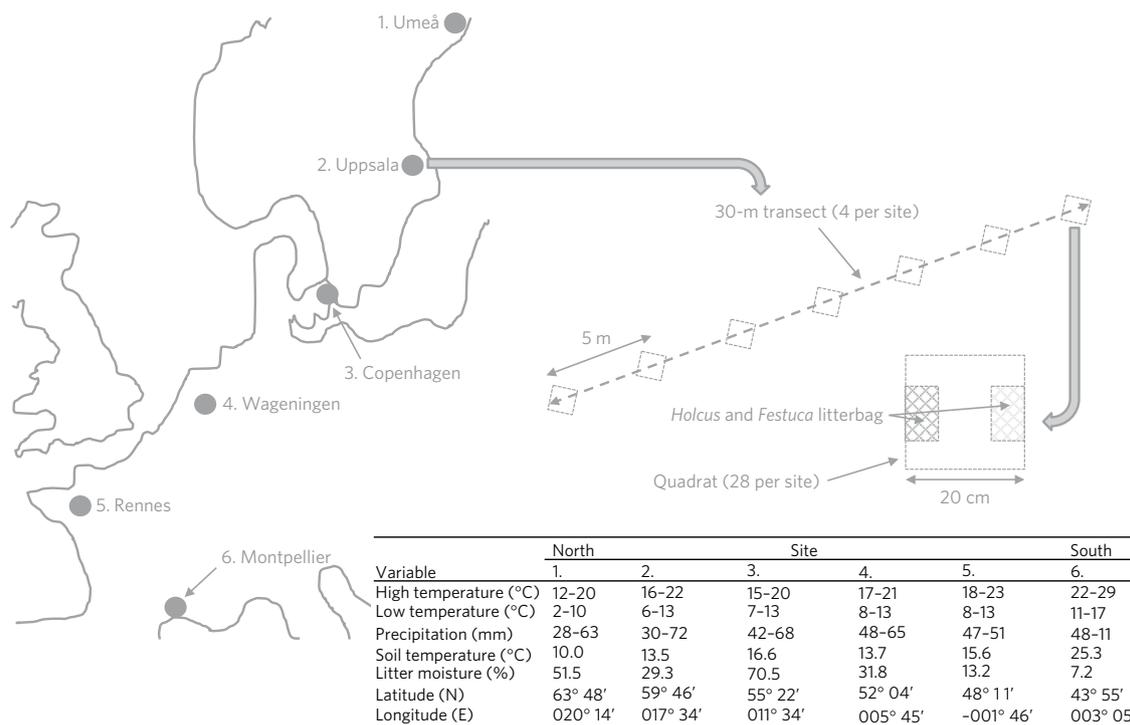


Fig. 1 | Study design and site characteristics. Spatial organisation and operational definitions of the study extent and observational grain are given in the hierarchical figure (site to microsite). Sites are named after the closest city and their climate data are from <https://climatedata.eu/> for the months (May–June) of litterbag incubation, giving the range across months in the average high and low temperature and precipitation. Soil data are the mean soil temperature and litter moisture data measured across the study period. Latitude and longitude data are for one transect at each site. Operational definitions of scale and variance: sites are shown as grey circles, microsites (that is, quadrats) are shown as squares. Broad scale: regional gradient, among sites (left); fine scale: local gradients, among quadrats at a site (right).

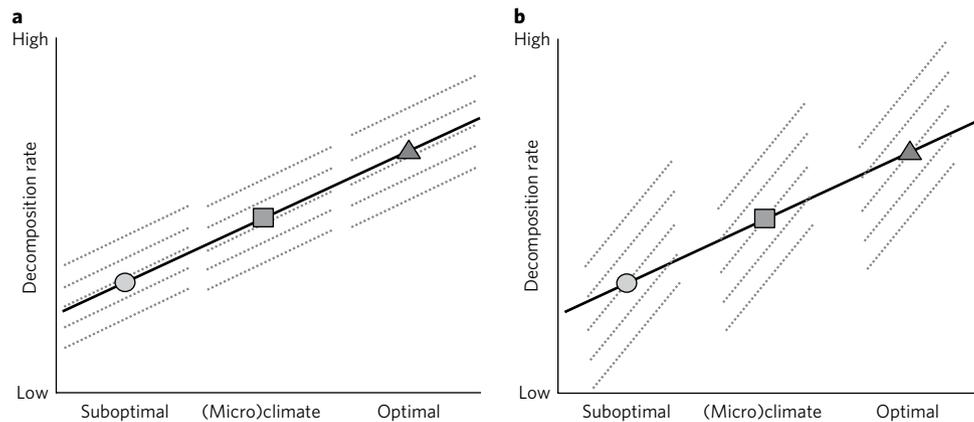


Fig. 2 | Competing assumptions for how decomposer communities affect relationships between climate and decomposition rates at regional to global scales. Ecosystem theory proposes that soil decomposer communities influence functional relationships between controls and decomposition rates in a spatially invariant manner. For example, broad-scale patterns among site-mean climate conditions are representative of a common relationship operating at finer spatial scales. **a**, We refer to such expectations as the assumption of scale invariance. The among-site mean climate process relationship (solid line) approximates the slope of the within-site microclimate-process relationships (dotted lines). Differences in absolute rates within sites can arise from differences in microclimate (change in rate for a single line) and values of other local controls (depicted by parallel dotted lines). In contrast to the expectations of the assumption of scale invariance, there is empirical evidence that decomposer communities can be functionally distinct, meaning that broad-scale patterns may instead emerge from distinct fine-scale (in this case within-site) relationships. **b**, We refer to this counter hypothesis as the assumption of scale dependence. Here, the among-site mean climate-process relationship (solid line) emerges from distinct relationships (dotted lines) operating within sites.

Table 1 | Coefficients, significance and r^2 values for the linear mixed-effect models used to evaluate controls on litter decomposition rates

Variables	Model				
	Microsite interactions		Unstandardized coefficients		
		Microsite interactions	Microsite main effects	Microclimate	Site-mean climate
Intercept	27.0 ± 0.689	-70.0 ± 14.629	-17.1 ± 6.264	-15.6 ± 6.365	-24.1 ± 6.960
Litter N	16.1 ± 0.856	45.3 ± 5.998	19.3 ± 1.173	19.2 ± 1.198	22.6 ± 1.283
Temperature	-4.49 ± 1.600	5.03 ± 1.344	1.05 ± 0.702	1.73 ± 0.681	2.81 ± 0.759
Temperature ²	-6.84 ± 3.285	-0.069 ± 0.033	-0.047 ± 0.018	-0.063 ± 0.018	-0.100 ± 0.021
Moisture	7.23 ± 1.256	0.240 ± 0.156	0.141 ± 0.023	0.120 ± 0.022	0.017 ± 0.028
Soil N	0.732 ± 1.075	0.151 ± 0.158	0.014 ± 0.028	NA	NA
Microbe	4.59 ± 1.165	4.70 ± 7.575	4.93 ± 1.477	NA	NA
Litter × temperature	-13.9 ± 1.888	-1.72 ± 0.233	NA	NA	NA
Litter × moisture	-0.275 ± 2.057	-0.007 ± 0.049	NA	NA	NA
Litter × soil N	1.58 ± 1.666	0.053 ± 0.056	NA	NA	NA
Litter × microbe	0.347 ± 1.997	0.535 ± 3.077	NA	NA	NA
Temperature × moisture	-7.03 ± 4.157	-0.014 ± 0.008	NA	NA	NA
Temperature × soil N	-3.09 ± 2.035	-0.009 ± 0.006	NA	NA	NA
Temperature × microbe	-1.46 ± 2.172	-0.185 ± 0.276	NA	NA	NA
Moisture × soil N	-3.02 ± 2.536	-0.002 ± 0.001	NA	NA	NA
Moisture × microbe	4.55 ± 2.923	0.111 ± 0.071	NA	NA	NA
Soil N × microbe	-0.409 ± 1.226	-0.014 ± 0.042	NA	NA	NA
Model r^2	66.3	66.3	57.1	55.2	57.6

The second column shows the standardized coefficients for the full model, where 'microsite' refers to the level at which the variables were observed, and 'interactions' to the inclusion of all two-way interactions among the predictors. Unstandardized coefficients were used when plotting Figs. 4, 5 and Supplementary Fig. 1. The consequence of aggregating microsite variation to generate 'site means' for the predictor variables was examined, but microsite variation in the response variable was retained to maintain the number of observations ($n = 303$). Significant ($P < 0.05$) and marginally significant ($P < 0.1$) coefficients are shown in bold and italic, respectively. Mean coefficients, their s.d. and significance are estimated using a Markov chain Monte Carlo sampling approach, and model r^2 values using a method that retains the random effects structure (see Methods). Model r^2 values were identical for the fixed and full (that is, fixed + random) effects. Note, in the standardized microsite interactions model, all square-root variance inflation factors (VIFs) were <2, except temperature², which was 2.98, and temperature × moisture, which was 2.30. In the unstandardized microsite interactions model, all VIFs were <10, except temperature, which was 16.0, and temperature² which was 14.8. In the unstandardized microsite main effects model, all square-root VIFs were <2, except temperature and temperature²; and the same was observed with the microclimate model, and the site-mean climate model. NA, not applicable.

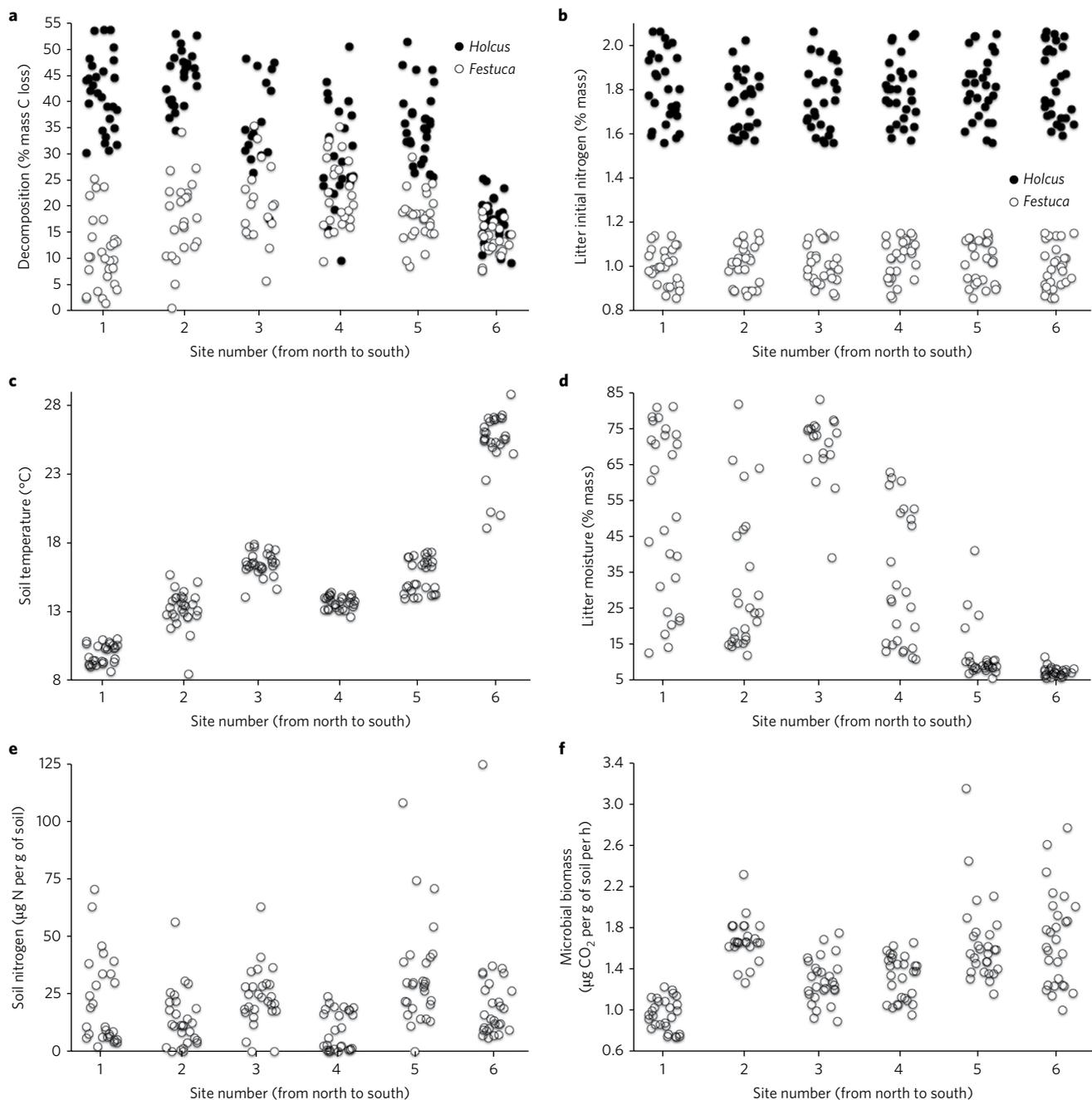


Fig. 3 | Measured variation in decomposition rates and controlling variables within and among sites. a–f, The response variable (decomposition) is shown in **a**, litter quality in **b**, climate variables in **c** and **d**, soil nitrogen availability in **e** and an estimate of the active decomposer biomass in **f**. Points represent individual observations ($n = 303$) and are jittered around the site number to prevent similar observations from obscuring one another. Sites are described in Fig. 1.

considerable variation, with loss rates for *Holcus* ranging from 7.72 to 53.7%, and for *Festuca* from 0.50 to 35.3%. Similarly, there was marked variation in the values of the climate controls, temperature and moisture, although they had contrasting within- versus among-site distributions. Soil temperatures clustered within sites, indicating that variation was much greater among sites (Fig. 3c), ranging from 10.0 to 25.3 °C for the most northern to southern site means. By contrast, microsite litter moisture only clustered around the site mean at the two most southern sites, where the mean site moisture was lowest (11.7 and 7.5%). At the most northern site the mean moisture was 51.6%, but varied among microsites from 12.8 to 81.3% (Fig. 3d). Microsite soil nitrogen (N) availability and

microbial biomass were more clustered than moisture, but within- versus among-site variation was still large (Fig. 3e,f). Soil N varied among sites from means of 9.0 to 32.8 µg N per g of soil, but varied within the most northern site alone from 2.3 to 70.6 µg N per g of soil. Equally, microbial biomass site means varied around 2-fold from 0.96 to 2.03 µg CO₂ per g of soil per h, but within sites, the microbial biomass varied from about 1.6-fold (the most northern site) to about 2.75-fold (the most southern site).

Prediction 1 proposes that emergent patterns between mean-site climate and decomposition might fail to capture relationships occurring at the microsite scale. We found no support for this prediction for temperature, in the ‘microclimate’ and ‘site-mean climate’

models (see Methods), given similar temperature coefficients (Table 1) and effect sizes (Fig. 4a). That is, the temperature–decomposition relationship was scale invariant (Fig. 1). This perhaps is not surprising, given that microsite soil temperature clustered around the site mean (Fig. 3c). Consequently the regional temperature–decomposition relationship should be, and was, approximately equivalent whether microsite or site-mean values were explored (Fig. 4a). There is evidence that microsite temperature can differ markedly from the site mean in some environmental contexts²². However across 60 sites spanning a broad range in eco-climatic conditions, Loeschner et al.²¹ found that microsite soil temperatures were representative of the site mean, suggesting that our finding that the temperature–decomposition relationship is scale invariant might generalize to numerous ecosystem types.

By contrast, the moisture–decomposition relationship was strongly scale-dependent: there was a pronounced moisture–decomposition relationship for the microsite model, but a weak one for the emergent pattern estimated from the site-mean model (Table 1, Fig. 4b). Specifically, across the large observed range of microsite moisture availability (5.7 to 83.2%), the site-mean model projected mass loss values ranging from a low of 27.4% to a high of 28.7%. By contrast, the microclimate model estimated a shift in decomposition across the same range of moisture from 23.9 to 33.2% mass loss (Fig. 4b). Site means therefore poorly captured regional heterogeneity in microsite moisture availability, generating a scale mismatch between the local mechanism and broad-scale pattern. Our data (Fig. 4b) therefore suggest that patterns emerging from among-site comparisons of site-mean moisture may fail to represent the causative relationships operating at the much finer spatial scales at which decomposer organisms respond to the environment. These findings raise questions about the use of site-mean (or coarser resolution) hydroclimatic data to parameterize ecosystem models. Overall, our data suggest that assumptions of the hierarchical model about scale invariance in climatic control are variable-dependent, cautioning

against its general application as a conceptual and numerical representation of controls on decomposition.

Using the microsite interactions model (see Methods), we evaluated prediction 2, which proposes that variables that are considered locally important should retain a strong influence at broad spatial scales. Following this prediction, the effect size of microbial biomass on decomposition rates was of a similar magnitude to those of the climatic variables (Fig. 5a). Specifically, estimated decomposition rates varied by around 16% mass C loss with temperature change, about 11% with moisture change and around 12% with microbial biomass change (Fig. 5a). Not surprisingly, given that we experimentally generated marked differences in litter quality, estimated mass loss increased approximately 24% (from 17 to 41%) with increasing initial litter N (Fig. 5a). By contrast, the soil N effect size was small, leading to an about 2% positive change in estimated mass C loss, but, as with all the other variables, the main effect coefficient was significant ($P < 0.05$; Table 1, Fig. 5a). Although some two-way interaction coefficients were of comparable or greater magnitude to the main effects of temperature, moisture and microbial biomass (Table 1), qualitatively the estimated effect sizes of these variables from the microsite interactions and microsite main effects models were similar (Figs. 4, 5b). That is, when interactions were removed, litter quality, temperature, moisture and microbial biomass all retained strong control of decomposition at the regional scale of our study (Table 1, Supplementary Fig. 1).

Exclusion of animal soil-decomposers does alter litter decomposition rates in at least some biomes^{15,24,26–28}, but microbial effects were not explicitly examined. However, the representation of microbial biomass or growth in biogeochemical models can improve predictive power^{9,29} and such variables are argued to relate most directly to spatial and temporal variation in biogeochemical processes rates^{7,8,30}. In support of these arguments, the absolute size of our estimated effects of microclimate on decomposition depended strongly on microbial biomass. Specifically, using the microsite

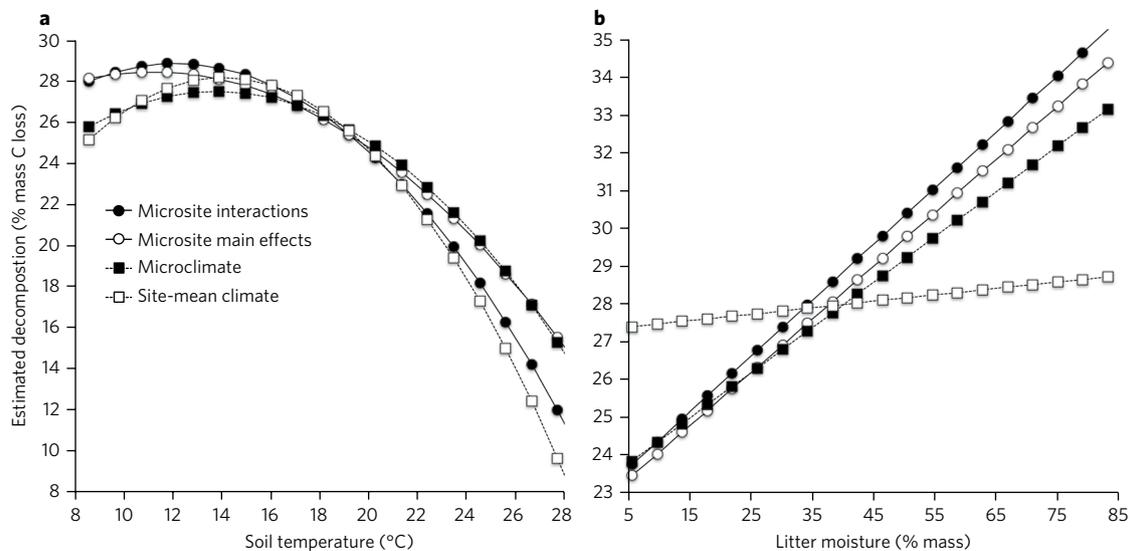


Fig. 4 | Estimated effects of temperature and moisture controls on decomposition rates. a, b, Effect sizes are estimated for temperature (a) and moisture (b) using the coefficients from the models presented in Table 1. Specifically, these coefficients were used in a regression equation, along with the mean value across all 168 quadrats for the controls that were not tested, and then for the control that was tested by systematically increasing the control from the lowest to highest observed values across the 168 quadrats. Comparisons of effect sizes between the microclimate versus site-mean climate models test whether patterns between site-mean climate and decomposition rates (effect sizes from the site-mean climate model) approximate those operating at the microsite scales at which decomposer organisms perceive the environment (effect sizes from the microclimate model). The temperature–decomposition relationship appears scale invariant, whereas the moisture–decomposition relationship is scale-dependent (Fig. 2). The two microsite models ask whether inclusion of microbial biomass and N availability as additional variables alters the estimated effects of temperature and moisture. Their inclusion does not appear to strongly affect the climate–decomposition relationships.

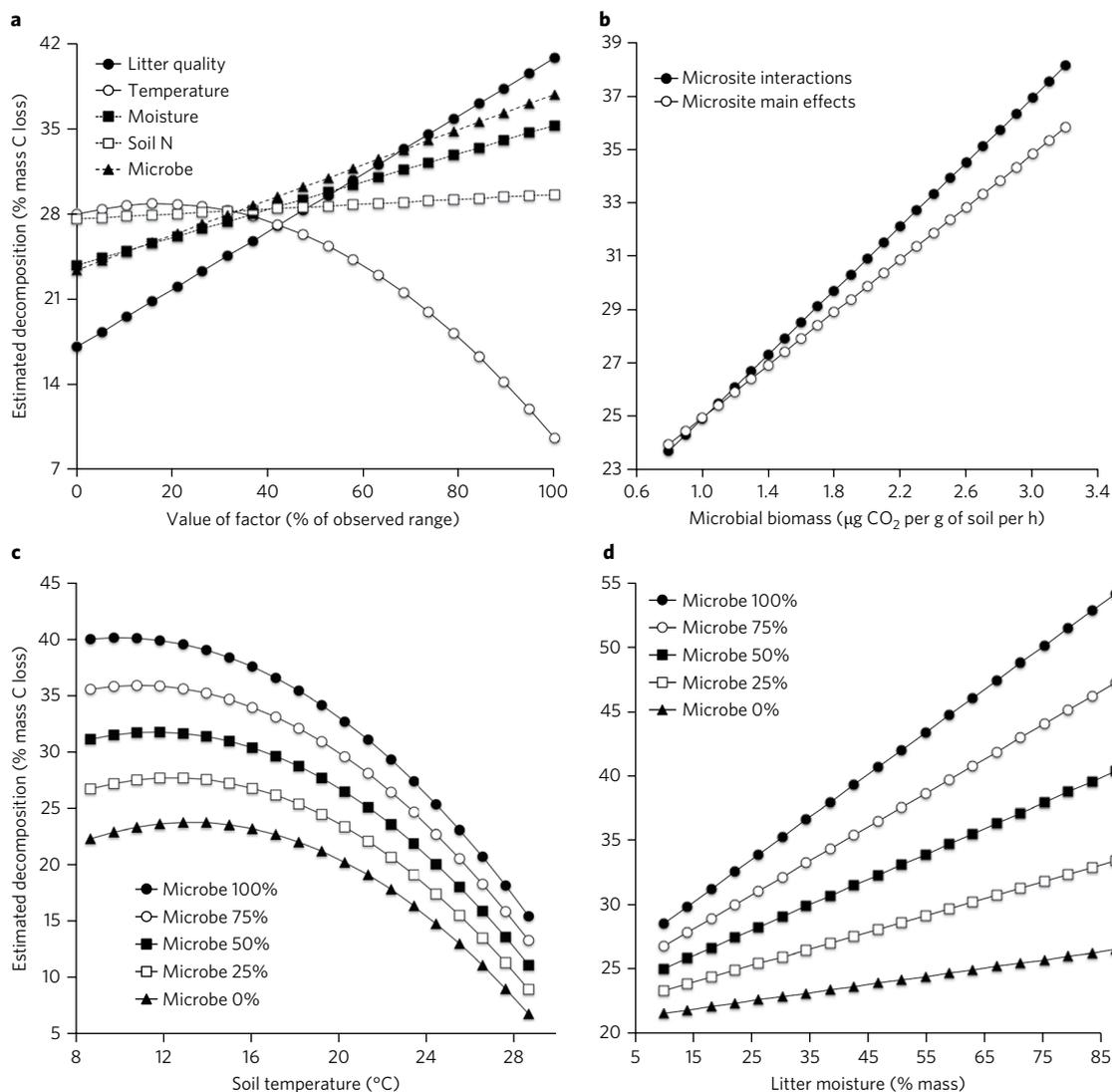


Fig. 5 | Estimated effects of controls on decomposition rates. Effect sizes are estimated from the microsite interactions model presented in Table 1, and in **b** also from the microsite main effects model, following the procedure described in the legend of Fig. 4. In **a**, the plots for each variable are generated using unstandardized coefficients from the microsite interactions model and the measured range in microsite conditions. The levels of each variable are relativized, ranging from the minimum (0%) to maximum (100%) measured value, revealing that microbial biomass (microbe) has an effect size approximately equivalent to both temperature and moisture. In **b**, the comparison of the two models asks whether the effect size of the microbial biomass is additively or non-additively dependent on the other controlling variables. Its effect seems primarily additive, given the similarity in the two plots. However, the effect sizes plotted in **c** and **d** reveal that this additive effect of microbial biomass can still strongly determine temperature and moisture effects on decomposition rates. The level of microbial biomass is relativized, with five values shown ranging from the minimum (0%) to maximum (100%) observed value. There are much stronger absolute decomposition responses to temperature and moisture when microbial biomass values are higher.

interactions model, we set microbial biomass at five values representing the observed range of microsite variation, and then varied temperature and moisture (Fig. 5c,d). Higher microbial biomass values generated a much greater absolute change in decomposition rates with increasing temperature or moisture (Fig. 5c,d). For example, estimated mass loss rates across the microsite moisture range only varied by around 5% in absolute terms when microbial biomass was low, to as much as around 25% (from 28.5 to 54.2% mass loss) when it was high. This influence of microbial biomass was primarily additive, given that, when it was dropped from the modelling (giving the microclimate model), there was minimal influence on the relative effect sizes of litter quality, temperature and moisture (Fig. 4, Table 1). A question that remains is whether the microbial traits selected by a site's climatic context^{17,18} in turn influence the magnitude of microclimate effects on decomposition, as is similarly

observed through climate selection of plant functional traits^{23,31}. Nevertheless, our data do support emerging numerical frameworks showing that explicit representation of microbes as controlling variables can markedly change expected effects of climate on broad-scale decomposition dynamics^{6,8,32}.

We found positive but relatively weak effects of soil N availability on decomposition (Supplementary Fig. 1), despite the fact that stoichiometry is considered a key control of microbial growth efficiencies and therefore biogeochemical process rates^{33–36}. The effects might have been stronger had the litter been of lower quality (for example, <1% initial N), requiring microbes to source N from the environment for growth and enzyme production³⁵. Such possibilities emphasize the fact that the effect sizes we report are specific to the spatial and temporal scale of our study. For example, the relative effect size of controls changes with how progressed litter

decay is^{37–39}. Future work will need to test whether the hierarchical model can approximate controls on later decomposition stages, in other biomes and at even broader spatial scales^{37,38}, when challenged with microsite data. Where the model cannot approximate controls (that is, where broad-scale emergent patterns do not reflect microsite relationships), new microsite-level studies will be needed to re-estimate parameter values for important controls. Such studies should test whether measuring fine-scale temporal as well as spatial variation might also necessitate a re-evaluation of how decomposition rates are controlled. Notably, our study leaves unresolved how microsite variation in litter quality might influence the nature of this co-dominant control. Furthermore, it suggests a need to re-design multi-site litter decomposition studies but does not address the challenge of making these studies practical given the very large number of observations that are apparently required to test when and to what extent emergent broad-scale patterns fail to capture microsite-level mechanisms¹.

We acknowledge that three aspects of our design may have influenced our findings: enclosing litter in mesh can alter the microclimate⁴⁰; the litter species do not occur at every site; and the microsite scale that we measured may also be mismatched with the litterbag scale of the response variable⁴¹. However, these caveats also apply to the multi-site litter decomposition experiments that have helped to build and reinforce the hierarchical model^{12–15}. The important caveat that we remove from these previous studies is the assumption that aggregate (that is, site-mean) data accurately capture the relationships between decomposition and the variables regulating it that operate at local (microsite) scales. Notably, there is growing evidence that C- and N-cycling processes in soil are driven to a large extent by microsite variation in controlling variables across landscape to regional scales^{42–44}. Those working in population and community ecology have wrestled with the insight that aggregate data may not represent local behaviour and can therefore lead to false conclusions and projections⁴⁵; it seems the same insight may need to be grappled with in ecosystem ecology.

Conclusions

Scaling theory in ecology describes how emergent patterns can arise from distinct and causative relationships operating at finer scales¹⁰. However, the issue is nested within a broader inferential challenge traditionally debated in the social sciences and increasingly so in the natural sciences^{42,46–48}. Although named without reference to the field of ecology, the issue is termed ‘ecological inference’ and refers to the process of using aggregate data to draw conclusions about individual-level behaviour⁴⁸. Causative relationships inferred from aggregate data often fail to represent the variables that control how individuals respond to and act on the environment⁴⁹. By comparison, relationships inferred from site-mean data in regional- to global-scale litter decomposition experiments may operate locally, or instead emerge from a set of distinct local-scale relationships and controlling variables. We have referred to these two possibilities as the assumption of scale invariance versus scale dependence (Fig. 2). Although we find that temperature control is scale invariant, our findings for moisture and microbial biomass control suggest that the hierarchical model may be the product of a logical inference fallacy. That is, it arises because aggregate data are falsely assumed to represent finer-scale causative relationships^{42,48,49}. Encouragingly, the rich body of work on scaling theory and the ecological inference fallacy⁵⁰ provides a platform for ecosystem ecology to test and potentially reformulate the conceptual and numerical models used to explain and predict how biogeochemical processes respond to a changing environment. Our findings help to reinforce calls to test and reconsider which environmental variables predominantly regulate biogeochemical process rates at regional to global scales, and when doing so, emphasize the need to work at the microsite scales at which organisms perceive the environment.

Methods

Experimental design. Site layout. Our research was conducted in grasslands spanning around 20° latitude in western Europe (Fig. 1). At each of the six study sites, we established four 30-m linear transects between 50 m and up to 2 km apart. Transects were chosen to capture within-site heterogeneity in microclimate and land-use intensity (for example, with or without grazing). Along each transect we established 20 × 20 cm quadrats at 5-m intervals, resulting in 7 quadrats per transect. In the context of this study, ‘quadrat’ serves as the ‘microsite scale’. Between 28 April and 16 May 2015, we placed two nylon mesh bags (5 × 10 cm; mesh size 0.9 × 1 mm) at each quadrat, approximately 10 cm apart. The mesh size presumably minimized the effect of larger soil fauna (for example, earthworms) on decomposition rates, and so our decomposition rates were probably primarily the product of microbes and micro- and mesofauna^{24,40}. Each mesh bag contained 1 g of air-dried grass foliar litter of either *Holcus lanatus* L. or *Festuca rubra* L., which differ in their litter chemical properties (see ‘Leaf litter’). This resulted in a total of 6 locations × 4 transects × 7 quadrats × 2 litter types = 336 litterbags. Litterbags were placed flush with the soil surface, within the existing litter layer and were retrieved after around 3 months. Of the 336 bags placed, 32 were lost in the field to such events as consumption by cows and accidental site mowing. The litter used to fill the litterbags was collected as freshly senesced material in grasslands local to the Dutch site.

Leaf litter. Mean litter properties for *H. lanatus* versus *F. rubra* were pH of 6.12 versus 5.61, N percentage of 1.78 versus 1.03, C:N of 24.7 versus 43.7, and lignin, calcium, magnesium and potassium contents of 157 versus 175, 3.72 versus 2.75, 1.31 versus 0.79, and 6.55 versus 1.50 mg g⁻¹, respectively. That is, regardless of the chemical property measured, *H. lanatus* was always less recalcitrant. By including the two contrasting litter types at every site, we generated equal within and among site variation in this variable. Doing so provided a statistical control whereby the strong within-site litter type effect should be approximated by the among-site effect, and so generate a scale invariant pattern (Fig. 2a). In addition, standardizing known controlling variables can improve estimated effects of the controls that were studied (for example, microclimate). Finally, litter traits are expected to interact with other variables, such as temperature²³, and so including this variable allowed us to test this possibility.

Measurements. Field. At each quadrat we determined the microclimate at the start, after around 6 weeks and at the end of the field incubation period. We collected three measurements per quadrat and time point of soil temperature at 5-cm depth using a hand-held thermometer. Such repeated spot measurements are effective at characterizing relative variation in microclimate⁴², and so our measurements are not indicative of absolute values experienced by the decomposing litters, but instead capture generally warmer versus cooler microsites, or drier versus wetter, across the course of the study. At the mid and end time point, soil moisture content was determined gravimetrically in three soil cores (5 cm depth, 2 cm diameter) from each quadrat; cores were pooled and dried at 105 °C until constant mass. We had intended to use these measurements (plus initial soil moisture) to estimate microsite moisture conditions, but marked differences in soil texture from clay (Umeå) to loamy sand (Wageningen) meant that soil gravimetric moisture was a poor surrogate for litter layer moisture conditions. Instead, we used litter moisture values (see ‘Testing prediction 1’). Additionally, at the start point of the field incubations, 8–10 soil cores of the same size were taken and pooled per quadrat and were used to determine soil gravimetric moisture, microbial biomass and N availability. Initial soil samples and retrieved litterbags were shipped to the Netherlands Institute of Ecology to ensure common processing. Collectively these measurements were intended to give estimates of four variables identified as important controls of decomposition either at broad scales (that is, temperature and moisture) or at local scales (that is, microbial biomass and N availability)^{32,35,51,52}. For soil microbial biomass, it is probably fairer to consider this an estimate of the spatial variation in soil community activity, which includes invertebrate decomposers, many of which will have been able to access the litter^{24,40}, and potentially also microbes not involved in litter decomposition.

Laboratory. Retrieved litter was cleaned of roots, fauna and soil, before the mass was determined fresh and after drying at 65 °C. It was next milled to a fine powder and analysed for total C content through elemental analysis (Flash 2000, Thermo-Fisher Scientific).

The initial 168 soils (6 locations × 4 transects × 7 quadrats) were passed through a 4-mm sieve and sub-sampled for gravimetric moisture, microbial biomass and N availability. We used the substrate-induced respiration method to estimate active microbial biomass⁵³, modified per ref. ⁵⁴. We estimated soil N availability by determining potential net N mineralization rates as the difference between salt-extractable N-NO₃⁻ and N-NH₄⁺ at time zero and after 14 days of incubation at 20 °C and 65% water-holding capacity⁵⁵. Soils were extracted with 1 M KCl and extracts were measured using an auto-analyser (QuAatro Segmented Flow Analyser; SEAL Analytical).

Initial litter properties were estimated using seven randomly collected samples per species, matching the sub-sampling for the litterbags. Total C and N content were measured as described above, lignin after a chloroform-methanol extraction

and hydrolysis with HCl, as previously described⁵⁶. Mineral nutrient concentrations and pH were measured following methods described in refs⁵⁷ and⁵⁸, respectively.

Data and inferential analysis. Overview of approach. We built a set of regression models, structured to represent and test between the assumptions of scale invariance and scale dependence in controls on litter decomposition (Fig. 2), to compare estimated effect sizes on decomposition for the four controlling variables that were studied. Specifically, we estimated the relative effect size of temperature, moisture, soil N availability and microbial biomass, across the range of observed values within and among our six sites. The relative effect size depends on the slope coefficient of the specific variable, the slope coefficient of any interaction it is involved in, and the range of observed values of the variable. We generated the coefficients by fitting linear mixed-effect models (LMMs). The effect size of a variable on mass C loss was estimated using these regression parameters, while holding all other variables constant (that is, the mean of all observations for each variable), and systematically varying the variable of interest across its measured range of values. That is, we plotted the regression equation for a model using the coefficients from the respective LMM, the mean value across all 168 quadrats for the controls that were not tested, and then for the tested control, we estimated decomposition rates by systematically increasing the value of the control from the lowest to highest observed value across the 168 quadrats.

The choice of variables to measure and then include in our statistical models (see 'Testing prediction 1') was based on the approach of ref.⁵⁹, which rejects model selection on philosophical and operational grounds. Philosophically, we investigated only variables for which the biological mechanism of their influence on decomposition is firmly established. Operationally, there is subjectivity and a lack of agreement in statistical model selection approaches, with different decisions leading to markedly different conclusions as to effect sizes. Instead, coefficients and therefore effect sizes are generally most robust when all terms are retained, assuming that each is included with a well-established biological foundation.

Testing prediction 1. Prediction 1 proposes that relationships between climate and decomposition rates should differ when site-mean versus microsite-level climate data are analysed. This prediction was evaluated by comparing whether temperature and moisture effects on mass C loss differed when the slope coefficients were estimated from microsite versus site-mean data. We established a single model structure to test prediction 1. It included only recognized broad-scale controls as variables (that is, temperature, moisture and litter type), but involved different data aggregation. The microclimate model was tested with observations of mass C loss for each litterbag and quadrat-level microclimate. The site-mean climate model was also run with all litterbag observations—to minimize changes in predictive power associated with changing values of n —but the values of the climate variables were the mean per site of the microclimate (that is, quadrat) observations. Therefore, in the microclimate model, the dataset had 168 unique temperature and moisture observations, whereas in the site-mean climate model, there were only six possible values (one per site) of temperature and moisture. Specifically, microsite control values were determined from the quadrat-level measurements, and site mean values were determined from the mean of the 28 quadrat-measurements within a site (that is, they were based on the exact same set of measurements). To account for potential spatial auto-correlation among the quadrats within a site, we fit a random error structure accounting for the spatial hierarchy in the design (quadrat nested within transect, with transect nested within site), assuming a common slope but spatially dependent intercept^{60,61}.

Similarly, litter type was included as the litterbag-level percentage N value, or as the mean percentage N per litter type, respectively (note that climate effect sizes were independent of how litter type was included). To determine a litterbag-level initial percentage N value, we randomly assigned to each litterbag a percentage N value (to the nearest 0.1%) drawn from the measured range of initial percentage N values from seven additional litterbag samples (Fig. 3b). We did this to acknowledge that there was variation among litterbags in initial percentage N and so using the mean initial percentage N would give a false account of the among-bag variation. For quadrat-level temperature, we calculated the mean soil temperature across the three field measurement periods. For quadrat-level moisture, given that soil gravimetric moisture was not useful given soil texture differences among sites, we calculated quadrat-level moisture as the mean of the *Holcus* and *Festuca* litterbag moisture values upon collection. We acknowledge that litters were probably drier at collection than at earlier points of the field incubations, given increasing temperatures and decreasing precipitation across the incubations, and so these values provide an estimate of relative spatial differences in moisture only. We used the mean across the two litter types, given that species-specific moisture values are often a product of leaf litter traits and are therefore correlated with litter quality¹.

Testing prediction 2. Prediction 2 proposes that any variable expected to be an important control at the microsite-level should have a strong effect when regional-scale patterns are analysed using microsite data. Specifically, we evaluated whether effect sizes of the soil microbial biomass and N availability variables had effect sizes comparable to recognized broad-scale controls (specifically temperature and moisture). We developed three model structures. The microsite interactions model included all variables (that is, temperature, moisture, microbial biomass and

N availability) and their two-way interactions. We included two-way interactions among the main effects given expectations that the relative effects of our variables should depend on one another. For example, the decomposition rate of more recalcitrant litters is expected to be more temperature sensitive^{61,62}. The microsite main effects model removed the two-way interactions to determine whether the effect sizes of the variables were primarily additive. The microclimate model was used again, but to evaluate whether dropping the soil microbial biomass and N availability terms altered inferences about temperature and moisture controls on mass C loss. Litter type (as initial percentage N) was again included in all models.

Statistical model specifics. The LMMs were fit with a Gaussian error distribution in the 'lme4' package of the R statistical program (version 3.1.3), using the 'lmer' function. Decomposition was calculated as the proportional mass C loss from the litterbags. Site, transect and quadrat were fit as random variables to the LMMs, with the finer-scale variables nested within the broader-scale variables, given the potential for autocorrelation caused by spatially clustering the litterbags⁶⁰. Before we tested the model structures described above, we tested the data distributions. A single and highly influential observation (based on Cook's D) was dropped from the dataset; it had a mass C loss value of 69.9%, far higher than any other observation (Fig. 3a), and markedly affected residual fits. The remaining data conformed to assumptions of normality, and a second-order temperature term was included given the observed unimodal relationship between temperature and mass loss. In addition, initial extractable N was a better choice (that is, a higher standardized coefficient) than potential N mineralization for soil N availability, and litter moisture (mean per quadrat) performed better than gravimetric soil moisture. Litter initial percentage N was used to represent litter quality given that it is a strong predictor of early-stage decomposition in grasses such as *H. lanatus*^{59,63}.

The square-root VIFs were <2 for the main effects, indicating low collinearity. As would be expected, there was a strong correlation between temperature and its second-order term, and where the effect of one variable strongly interacted with another. We reduced these VIF values by standardizing the observed value of each variable by subtracting the mean and dividing by two standard deviations⁶⁴. The resulting standardized coefficients also permit coefficients to be directly compared for variables measured on different unit scales. Confirming the validity of our inferences in spite of introduced collinearity when second-order terms and interactions were permitted, variables with large effect sizes calculated on the basis of the unstandardized coefficients also had large standardized coefficients. In addition, in the microsite main effects model all two-way interactions were dropped, removing collinearity and concerns about over-fitting, and the relative magnitude of the coefficients were mostly unchanged (Table 1).

All reported P values are quasi-Bayesian but retain the same interpretation as frequentist P values⁶⁵. We considered coefficients with $P < 0.05$ to be significant and coefficients with $P < 0.10$ to be marginally significant. We calculated the r^2 values for each model following ref.⁶⁶. Calculation of r^2 values is common practice when modelling decomposition and a high value associated with a specific explanatory variable is often associated with that variable having a strong effect size. This reasoning makes no sense within the context of our study, because litter type was experimentally controlled and accurately measured within and among sites, whereas the other variables relied on observed variation and measurements that represented—but probably did not fully characterize—the conditions that acted on decomposer activity. The latter conditions make data more 'noisy', lowering r^2 values, but this, in the absence of systematic bias, will not change the coefficient estimates and therefore effect sizes¹. We therefore only report the r^2 value for each model, to verify that they had the potential to explain a substantive degree of the variance in decomposition rate.

Life Sciences Reporting Summary. Further information on experimental design and reagents is available in the Life Sciences Reporting Summary.

Data availability. Experimental data that support these findings and the R code for the statistical models are available via the Dryad Digital Repository (<https://doi.org/10.5061/dryad.c44h0>).

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Author contributions

M.A.B. and G.F.V. designed the study, co-wrote the manuscript, constructed litterbags and carried out the lab analyses. All authors established, maintained and collected data from the field sites. M.A.B., G.F.V., D.S.M. and S.A.W. analysed data. All authors contributed to data interpretation and writing of the paper.

Competing interests

The authors declare no competing financial interests.

Additional information

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▶ Experimental design

1. Sample size

Describe how sample size was determined.

Six sites were chosen given that six points or more along a continuous axis is considered to give much greater power (with regression designs) than 5 points or less. However, given that each observation was unique, there were 336 observations with 168 unique values for the climate gradient predictor variables. This equated to 28 replicates of each sample type per site, a number that was based on previous experimental work (Bradford et al. 2016 Journal of Ecology) that found that using 20 to 25 sample units per site gave high statistical power, with this power increase flattening out at about 20 replicates.

2. Data exclusions

Describe any data exclusions.

One data point was excluded. It was found in formal outlier tests to have very strong influence on the data distributions, with a much higher value than the other 335 observations. The exclusion is fully explained in the manuscript.

3. Replication

Describe whether the experimental findings were reliably reproduced.

The paper reports a single experiment but with high within-site replication, adding high confidence to the observed differences as reflected by low error around large coefficients and strongly significant p values

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

We used a stratified random design within and among sites, which is more powerful than a simply random design given that we deliberately exploited natural variation to generate a broad distribution in the values of our predictor variables. Within each stratification, sample units were randomly assigned.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Investigators were not blinded because one group was responsible for the set-up, maintenance, and harvesting of one of the six sites. But the design was replicated at each site and exploited natural variation, so individual investigators were working "blind" as to the assumptions of other site investigators as to the most important variables. Further, samples were all processed at a single laboratory and the technicians processing samples were blind to where samples came from. They were simply given a number that related to a sample, and did not know the source location. That is, lab measurements were carried out blind of the field assignments of specific samples.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

The statistical freeware R

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). [Nature Methods guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All materials are readily available

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

not applicable

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

not applicable

b. Describe the method of cell line authentication used.

not applicable

c. Report whether the cell lines were tested for mycoplasma contamination.

not applicable

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

not applicable

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

not applicable

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

not applicable