Testing 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 CO_2. 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. Further, 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 posits that the primary controls on the rate of decomposition are climate, litter quality and decomposer organisms[1]. 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 whose effect is negligible at broader scales[5]. Consequently decomposers have been omitted as controls

from biogeochemical models, whereas 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 lack of evidence is suggested by scaling theory, where the influence of mechanisms that act locally can be obscured in emergent, broad-scale patterns[10].

Pattern and scale has been described as the central issue in ecology, where the inherent challenge to prediction and understanding lies in the elucidation of mechanisms, which commonly operate at different scales to those on which the patterns are observed[10]. This scale mismatch appears true for at least some ecosystem processes, such as plant productivity[10,11]. Decomposition processes, also, are controlled by variables operating at finer scales than those at which the variables are typically measured and evaluated[1]. 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 collecting few observations (typically 2 to 4 per site per litter species per collection) – from which a mean decomposition rate is determined – and also use of site-mean data to estimate climatic controls[1]. Yet the hierarchical model, and its representation in the structure of biogeochemical models, is based on the assumption that controls act at the microsite level, by regulating the activities of decomposer organisms[5,16]. That is, the hierarchical model is conceptually grounded in local (i.e. microsite) dynamics, but has been developed and substantiated with site-mean data that represents climate control of decomposition as an among-site relationship.

Understanding controls on litter decomposition across regional scales is then necessarily intertwined with scaling theory. This body of theory[10] suggests that broad-scale patterns might emerge from distinct, local-scale causative relationships, which 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. The first is that the activities of decomposer communities are shaped by environmental selection for a subset of functional traits, which then uniquely dictate how decomposition rates respond to changing climatic controls[17-20]. The second is that microclimate can vary widely within a site[21,22]. As such, site-mean climate data are likely a poor surrogate for the range in microclimate experienced by decomposer organisms within a site[21]. 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 use a multi-site, litter decomposition study to test between the competing assumptions of scale invariance and dependence (Figs. 1,2). We worked across a climate gradient in Europe at six grassland sites spanning boreal climate in northern Sweden to Mediterranean climate in southern France. We predicted 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 under study. In addition, litter traits are expected to interact with controls such as temperature[23] and so including this variable allowed us to 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 3-month field incubations was approximately twice as great for the higher quality *Holcus* litter (33.8±11.62%; mean±SD) than for the *Festuca* litter (16.8±7.15%). However, there was 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, meaning 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. In contrast, microsite litter moisture only clustered around the site mean at the two most southern sites, where 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 (Figs. 3e,f). Soil N varied among sites from means of 9.0 to 32.8 μg N g soil^{-1} but within the most northern site alone from 2.3 to 70.6 μg N g soil^{-1}. Equally, microbial biomass site means varied ~2-times from 0.96 to 2.03 μg CO_2 g soil^{-1} h^{-1}, but within sites from about 1.6-times (most northern) to about 2.75-times (most southern).

Prediction 1 was 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, with the "Microclimate" and "Site-mean climate" models (see Methods) giving 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 to the site mean in some environmental contexts[22]. However across 60 sites spanning a broad range in eco-climatic conditions, Loescher *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.

In 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%. In contrast, the Microclimate model estimated a shift in decomposition across the same range in 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 local mechanism and broad-scale pattern. Our data (Fig. 4b) consequently suggest that patterns emerging from among-site comparisons of site-mean moisture may fail to represent 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 parameterise 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 that variables 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 similar magnitude to those for the climatic variables (Fig. 5a). Specifically, estimated decomposition rates varied by ~16% mass C loss with temperature change, ~11% with moisture change, and ~12% with microbial biomass change (Fig. 5a). Not surprisingly, given that we experimentally generated marked differences in litter quality, estimated mass loss increased ~24% (from 17 to 41%) with increasing initial litter N (Fig. 5a). The soil N effect size was by contrast small, leading to about a 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 2-way interaction coefficients were of comparable or greater magnitude to the main effects for 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 on decomposition at the regional scale of our study (Table 1, Supplementary Fig. 1).

Exclusion of soil animal 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 process 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 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 ~5% in absolute terms when microbial biomass was low, to as much as ~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). An outstanding question 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 dramatically 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 stoichiometry is considered a key control on microbial growth efficiencies and hence biogeochemical process rates[33-36]. The effects might have been stronger had the litter been of lower quality (e.g. <1% initial N), requiring microbes to source N from the environment for growth and enzyme production[35]. 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 (i.e. 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. Further, 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 apparently required to test when and to what extent emergent broad-scale patterns fail to capture microsite-level mechanisms[1].

We acknowledge that three aspects of our design may have influenced our findings: enclosing litter in mesh can alter the microclimate[40]; the litter species do not occur at every site; and the microsite scale we measured may also be mismatched with the litterbag scale of the response variable[41]. However, these caveats also apply to the multi-site litter decomposition experiments that have helped build and reinforce the hierarchical model[12-15]. The important caveat that we remove from these previous studies is the assumption that aggregate (i.e. 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 hence lead to false conclusions and projections[45]; 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-scales[10]. 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 apparently 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 behaviour[48]. Causative relationships inferred from aggregate data often fail to represent the variables that control how individuals respond to and act on the environment[49]. By comparison, relationships inferred from site-mean data in regional- to global-

242 scale litter decomposition experiments may operate locally, or instead emerge from a set of
243 distinct local-scale relationships and controlling variables. We have referred to these two
244 possibilities as the assumption of scale invariance versus scale dependence (Fig. 2). Although we
245 find temperature control scale invariant, our findings for moisture and microbial biomass control
246 suggest that the hierarchical model may be the product of a logical inference fallacy. That is, it
247 arises because aggregate data are falsely assumed to represent finer-scale causative
248 relationships[42,48,49]. Encouragingly, the rich body of work on scaling theory and the ecological
249 inference fallacy[50] provides a platform for ecosystem ecology to test and potentially reformulate
250 its conceptual and numerical models used to explain and predict how biogeochemical processes
251 respond to a changing environment. Our findings help reinforce calls to test and reconsider
252 which environmental variables predominantly regulate biogeochemical process rates at regional-
253 to global-scales, and when doing so emphasize the need to work at the microsite scales at which
254 organisms perceive the environment.
255
256 **Methods**
257 **Experimental design.** *Site layout.* Our research was conducted in grasslands spanning ~20°
258 latitude in Western Europe (Fig. 1). At each of six study sites, we established four 30-m linear
259 transects between 50 m and up to 2 km apart. Transects were chosen to capture within-site
260 heterogeneity in microclimate and land-use intensity (e.g. with or without grazing). Along each
261 transect we established 20×20 cm quadrats at 5-m intervals, resulting in 7 quadrats per transect.
262 In the context of this study, ‘quadrat’ serves as the ‘microsite scale’. Between 28 April and 16
263 May 2015, we placed two nylon mesh bags (5×10 cm; mesh size 0.9×1 mm) at each quadrat, ~10
264 cm apart. The mesh size presumably minimized the effect of larger soil fauna (e.g. earthworms)

on decomposition rates, and so our decomposition rates were likely primarily the product of microbes and micro- and mesofauna[24,40]. Each mesh bag contained 1 g air-dried grass foliar litter of either *Holcus lanatus* L. or *Festuca rubra* L., which differ in their litter chemical properties (see below). 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 ~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 vs. 5.61, %N of 1.78 vs. 1.03, C:N of 24.7 vs. 43.7, and lignin, calcium, magnesium and potassium contents of 157 vs. 175, 3.72 vs. 2.75, 1.31 vs. 0.79, and 6.55 vs. 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). Second, standardizing known controlling variables can improve estimated effects of the controls under study (e.g. microclimate). Third, litter traits are expected to interact with other variables, such as temperature[23], and so including this variable allowed us to test this possibility.

Measurements. *Field.* At each quadrat we determined microclimate at the start, after ~6 weeks and at the end of the field incubation period. We collected three measures 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 microclimate[42], and so our measures are not indicative of absolute values experienced by the decomposing litters but instead capture generally warmer vs. cooler microsites, or drier vs. 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 diam.) from each quadrat; cores were pooled and dried at 105°C until constant mass. We had intended to use these measures (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* below). 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 measures were intended to give estimates of four variables identified as important controls of decomposition either at broad-scales (i.e. temperature and moisture), or at local-scales (i.e. 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.

309 *Laboratory.* Retrieved litter was cleaned of roots, fauna and soil, before mass was determined
310 fresh and after drying at 65°C. It was next milled to a fine powder and analysed for total C
311 content through elemental analysis (Flash 2000, Thermo Fisher Scientific, Bremen, Germany).
312 The initial 168 soils (6 locations × 4 transects × 7 quadrats) were passed through a 4-mm
313 sieve and sub-sampled for gravimetric moisture, microbial biomass and N availability. We used
314 the substrate-induced respiration (SIR) method to estimate active microbial biomass[53], modified
315 per Fierer *et al.*[54]. We estimated soil N availability by determining potential net N mineralization
316 rates as the difference between salt-extractable $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ at time zero and after 14 d of
317 incubation at 20°C and 65% water holding capacity[55]. Soils were extracted with 1M KCl and
318 extracts measured using an auto-analyser (QuAAtro Segmented Flow Analyser; SEAL
319 Analytical; Norderstedt, Germany).
320 Initial litter properties were estimated using seven randomly collected samples per
321 species, matching the sub-sampling for the litterbags. Total C and N content were measured as
322 described above, lignin after a chloroform/methanol extraction and hydrolysis with HCl,
323 following Poorter & Villar[56]. Mineral nutrient concentrations and pH were measured following
324 methods described in Hendry and Grime[57] and Cornelissen *et al.*[58], respectively.
325
326 **Data and inferential analysis.** *Overview of approach.* We built a set of regression models,
327 structured to represent and test between assumptions of scale invariance versus dependence in
328 controls on litter decomposition (Fig. 2), to compare estimated effect sizes on decomposition of
329 the four controlling variables under study. Specifically, we estimated the relative effect size for
330 temperature, moisture, soil N availability and microbial biomass, across the range of observed
331 values within and among our six sites. The relative effect size depends on the slope coefficient

for the specific variable, the slope coefficient for 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 (i.e. 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 not under test, and then for the control under test we estimated decomposition rates by systematically increasing the value of the control from the lowest to highest observed values across the 168 quadrats.

The choice of variables to measure and then include in our statistical models (described next) was based on the approach of Hobbs *et al.*[59], which rejects model selection on philosophical and operational grounds. Philosophically, we investigated only variables where biological mechanism as to their influence on decomposition is firmly established. Operationally, there is subjectivity and lack of agreement in statistical model selection approaches, with different decisions leading to markedly different conclusions as to effect sizes. Instead, coefficients and hence effect sizes are generally most robust when all terms are retained, assuming that each is included with well-established biological foundation.

Testing Prediction 1. Prediction 1 was 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

355 established a single model structure to test Prediction 1. It included only recognized broad-scale
356 controls as variables (i.e. temperature, moisture and litter type), but involved different data
357 aggregation. The “Microclimate” model was tested with observations of mass C loss for each
358 litterbag and quadrat-level microclimate. The “Site-mean climate” model was also run with all
359 litterbag observations – to minimize changes in predictive power associated with changing
360 values of *n* – but the values of the climate variables were the mean per site of the microclimate
361 (i.e. quadrat) observations. Hence in the Microclimate model the dataset had 168 unique
362 temperature and moisture observations, whereas in the Site-mean climate model there were only
363 six possible values (one per site) of temperature and moisture. Specifically, microsite control
364 values were determined from the quadrat-level measures, and site mean values determined from
365 the mean of the 28 quadrat-measures within a site (i.e. they were based on the exact same set of
366 measurements). To account for potential spatial auto-correlation among the quadrats within a
367 site, we fit a random error structure accounting for the spatial hierarchy in the design (quadrat
368 nested within transect, with transect nested within site), assuming a common slope but spatially-
369 dependent intercept[50,60].

370 Similarly, litter type was included as the litterbag-level %N value, or as the mean %N per
371 litter type, respectively (note that climate effect sizes were independent of how litter type was
372 included). To determine a litterbag-level initial %N value, we randomly assigned to each
373 litterbag a %N value (to the nearest 0.1%) drawn from the measured range of initial %N values
374 from seven additional litterbag samples (Fig. 3b). We did this to acknowledge that there was
375 variation among litterbags in initial %N and so using the mean initial %N would give a false
376 account of the among-bag variation. For quadrat-level temperature, we calculated the mean soil
377 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 on collection. We acknowledge that litters were probably drier at collection than at earlier points of the field incubations, given increasing temperatures and declining 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 thus correlated with litter quality[4].

Testing Prediction 2. Prediction 2 was 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 (i.e. temperature, moisture, microbial biomass, N availability) and their 2-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 2-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 %N) was again included in all models.

Statistical model specifics. The LMMs were fit with a Gaussian error distribution in the "lme4" package for 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 litterbags[60]. 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. Also, initial extractable N was a better choice (i.e. 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 %N was used to represent litter quality given that it is a strong predictor of early-stage decomposition in grasses such as *H. lanatus*[39,63].

The square-root variance inflation factors (vif) 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 deviations[64]. 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 2-way interactions were dropped, removing collinearity and concerns about over-fitting, and the relative magnitude of the coefficients were largely unchanged (Table 1).

All reported *P*-values are quasi-Bayesian but retain the same interpretation as frequentist *P*-values[65]. We considered coefficients with $P<0.05$ to be significant and coefficients with $P<0.10$ marginally significant. We calculated the r^2 values for each model following Nakagawa and Schielzeth[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 likely did not fully characterize – the conditions that acted on decomposer activity. The latter conditions make data more "noisy", lowering r^2 values, but in the absence of systematic bias will not change the coefficient estimates and hence effect sizes[1]. We therefore only report the r^2 value for each model, to verify they had the potential to explain a substantive degree of the variance in decomposition rate.

Data availability

Experimental data in the support of these findings and the R code for the statistical models are available via the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.c44h0).

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605
606 **Authorship**
607 MAB and GFV contributed equally to this work. They designed the study, co-wrote the
608 manuscript, constructed litterbags and carried out the lab analyses. All authors established,
609 maintained and collected data from the field sites. MAB, GFV, DSM and SAW analysed data.
610 All authors contributed to data interpretation and paper writing.
611
612 **Additional information**
613 **Supplementary information** is available for this paper.
614 **Correspondence and requests for materials** should be addressed to M.A.B.
615
616 **Competing interests**
617 The authors declare no competing financial interests.
618
619 **Figure 1 | Study design and site characteristics.** Spatial organisation and operational
620 definitions of the study extent and observational grain are given in the hierarchical figure (site to
621 microsite). Sites are named for the closest city and their climate data are from climatedata.eu for
622 the months (May-June) of litterbag incubation, giving the range across months in the average
623 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 in each site.

Figure 2 | Competing assumptions for how decomposer communities affect relationships between climate and decomposition rates at regional to global scales. Ecosystem theory holds 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): the assumption of *scale invariance*. Increasingly 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): the assumption of *scale dependence*.

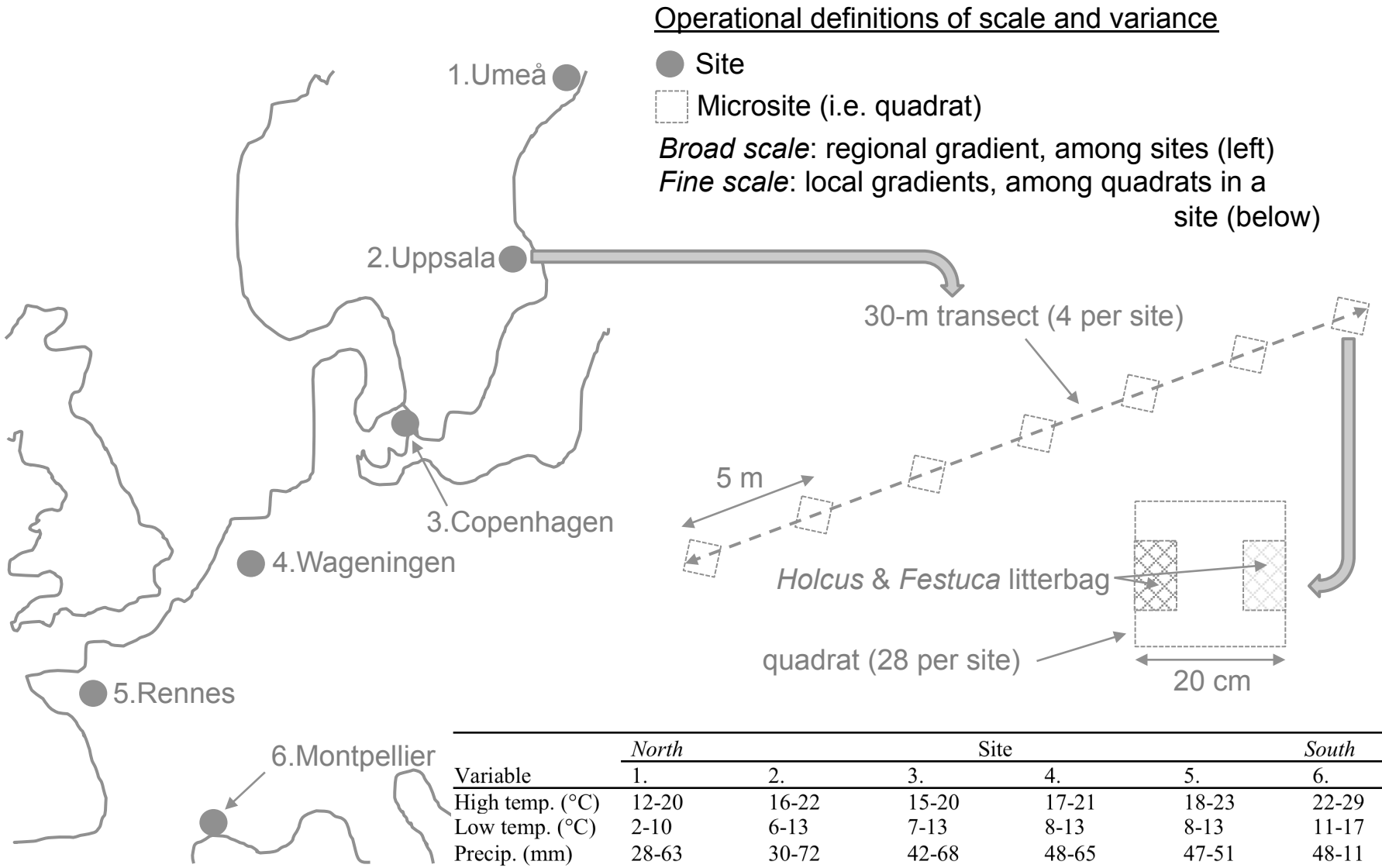
Figure 3 | Measured variation in decomposition rates and controlling variables within and among sites. The response variable (decomposition) is shown in (a), litter quality in (b), climate variables in (c) and (d), soil nitrogen availability in (e) and an estimate of the active decomposer biomass in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

Figure 4 | Estimated effects of temperature and moisture controls on decomposition rates. 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 not under test, and then for the control under test 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

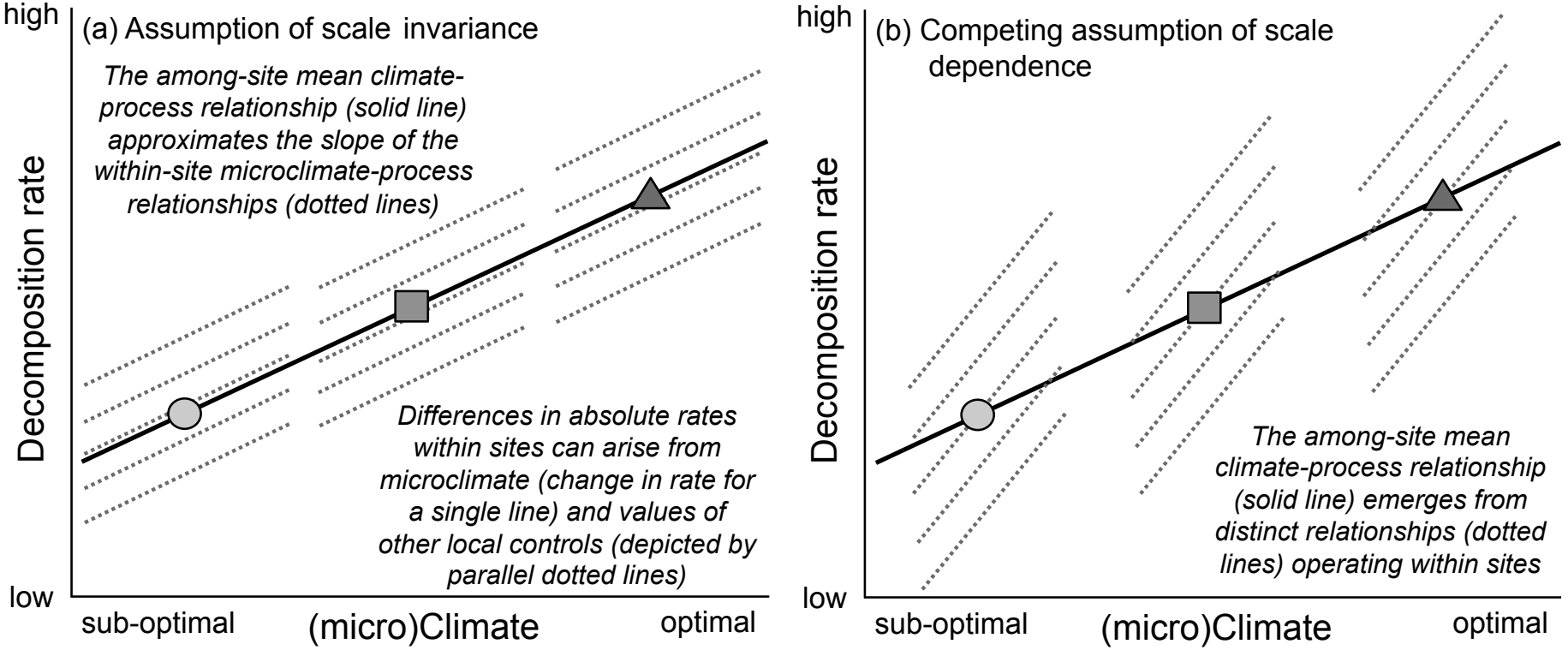
rates (effect sizes from the Site-mean climate model) approximate those operating at the microsite scales at which decomposer organisms perceive the environment (effect sizes from the Microclimate model). The temperature-decomposition relationship appears scale invariant whereas the moisture-decomposition 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.

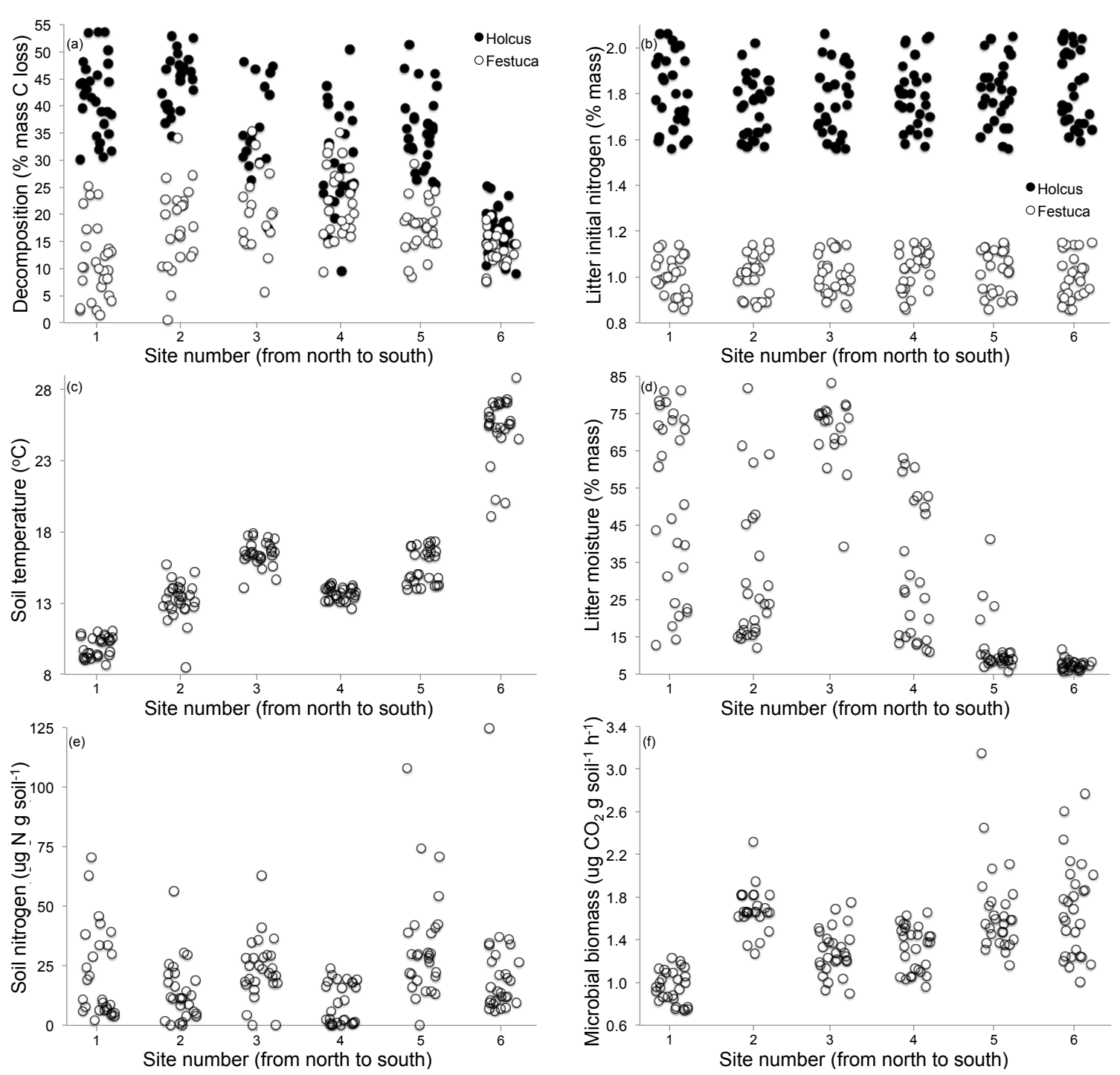
Figure 5 | Estimated effects of controls on decomposition rates. Effect sizes are estimated from the Microsite interactions model presented in Table 1, and in (b) also from the Microsite main effects model, following the procedure described in the legend of Fig. 4. In (a), plots for each variable are 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), comparison of the two models asks whether the effect size of the microbial biomass is additive 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 greater.

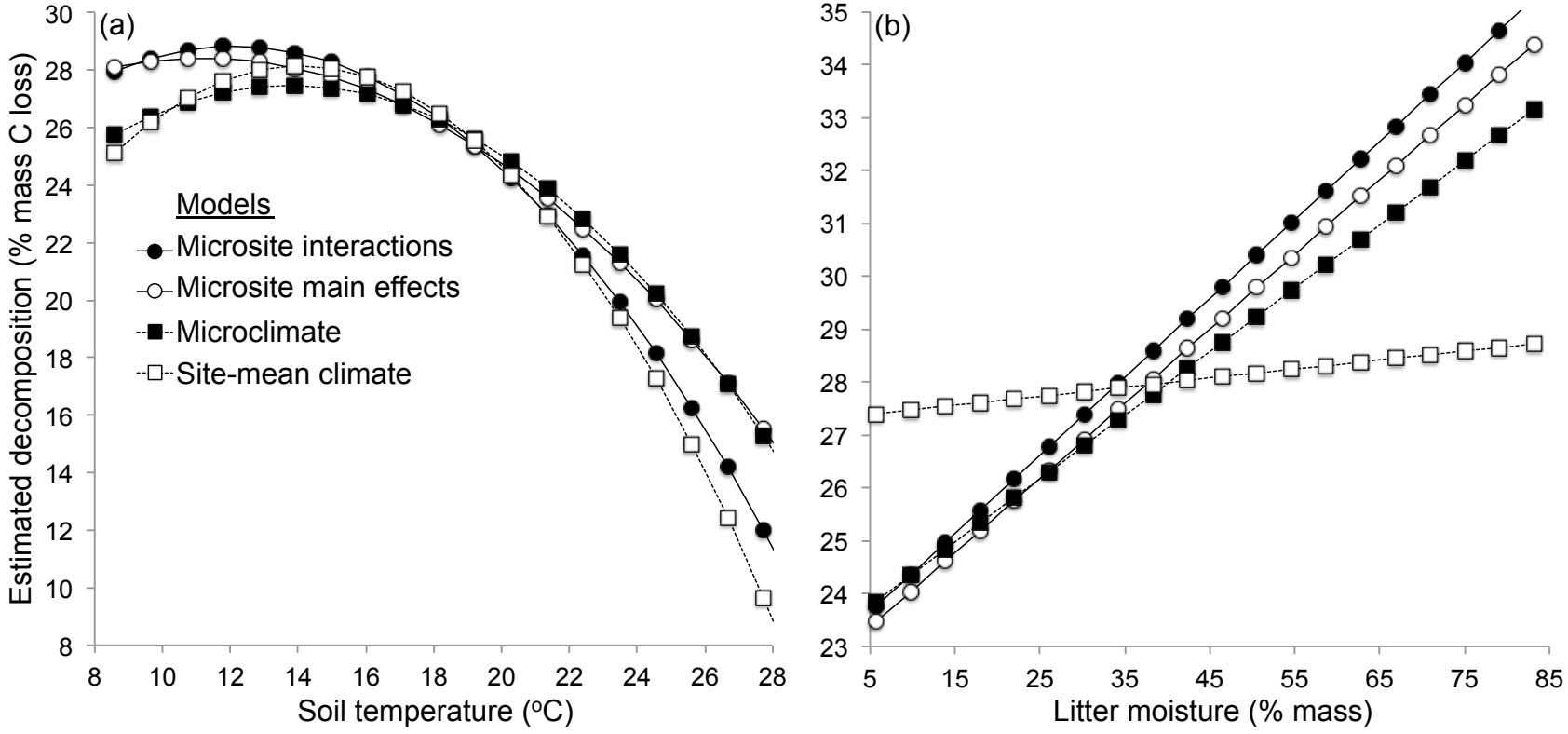
Table 1 | Coefficients, significance and r^2 values for the linear mixed models used to evaluate controls on litter decomposition rates.



	North					*South*
	Site					
Variable	1.	2.	3.	4.	5.	6.
High temp. (°C)	12-20	16-22	15-20	17-21	18-23	22-29
Low temp. (°C)	2-10	6-13	7-13	8-13	8-13	11-17
Precip. (mm)	28-63	30-72	42-68	48-65	47-51	48-11
Soil temp. (°C)	10.0	13.5	16.6	13.7	15.6	25.3
Litter moist. (%)	51.5	29.3	70.5	31.8	13.2	7.2
Latitude (N)	63°48'	59°46'	55°22'	52°04'	48°11'	43°55'
Longitude (E)	020°14'	017°34'	011°34'	005°45'	-001°46'	003°05'


high
(a) 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)
Decomposition rate
Differences in absolute rates within sites can arise from microclimate (change in rate for a single line) and values of other local controls (depicted by parallel dotted lines)
low
sub-optimal
(micro)Climate
optimal
high
(b) Competing assumption of scale dependence
Decomposition rate
The among-site mean climate-process relationship (solid line) emerges from distinct relationships (dotted lines) operating within sites
low
sub-optimal
(micro)Climate
optimal


(a)
Holcus
Festuca
Decomposition (% mass C loss)
Site number (from north to south)
(b)
Litter initial nitrogen (% mass)
Site number (from north to south)
(c)
Soil temperature (°C)
Site number (from north to south)
(d)
Litter moisture (% mass)
Site number (from north to south)
(e)
Soil nitrogen (ug N g soil-1)
Site number (from north to south)
(f)
Microbial biomass (ug CO2 g soil-1 h-1)
Site number (from north to south)


(a)
(b)
Estimated decomposition (% mass C loss)
Soil temperature (°C)
Litter moisture (% mass)
Models
Microsite interactions
Microsite main effects
Microclimate
Site-mean climate

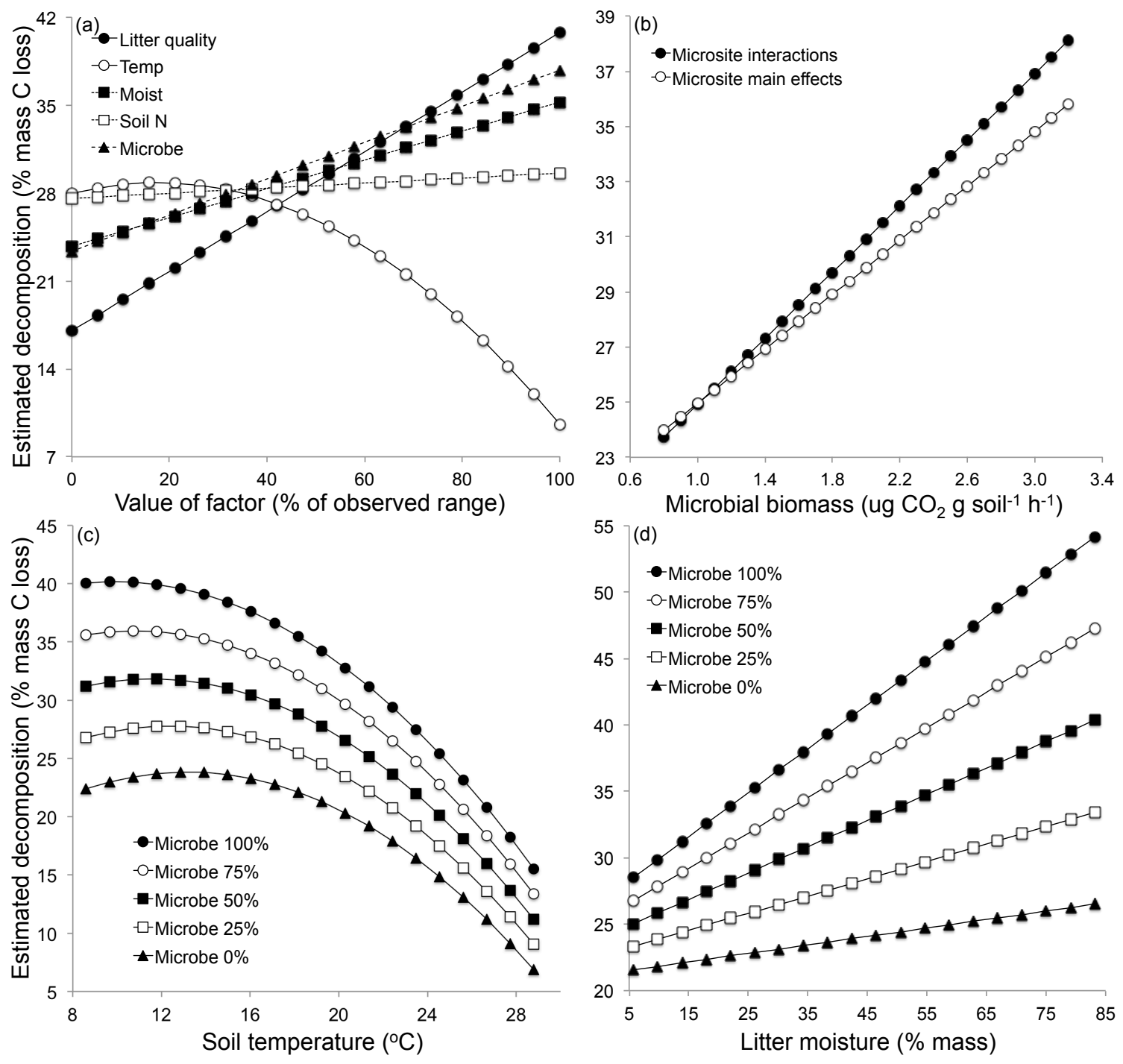

(a)
Litter quality
Temp
Moist
Soil N
Microbe
Estimated decomposition (% mass C loss)
Value of factor (% of observed range)
(b)
Microsite interactions
Microsite main effects
Microbial biomass (ug CO2 g soil-1 h-1)
(c)
Microbe 100%
Microbe 75%
Microbe 50%
Microbe 25%
Microbe 0%
Estimated decomposition (% mass C loss)
Soil temperature (ºC)
(d)
Microbe 100%
Microbe 75%
Microbe 50%
Microbe 25%
Microbe 0%
Litter moisture (% mass)

Table 1 Coefficients, significance and r^2 values for the linear mixed models used to evaluate controls on litter decomposition rates[1]. Shown in the second column are 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 2-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 fonts, respectively.

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**
Temp^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
Lit ×Temp	**-13.9±1.888**	**-1.72±0.233**	na	na	na
Lit × Moist	-0.275±2.057	-0.007±0.049	na	na	na
Lit × soilN	1.58±1.666	0.053±0.056	na	na	na
Lit × Mic	0.347±1.997	0.535±3.077	na	na	na
Temp × Moist	*-7.03±4.157*	*-0.014±0.008*	na	na	na
Temp × soilN	*-3.09±2.035*	*-0.009±0.006*	na	na	na
Temp × Mic	*-1.46±2.172*	*-0.185±0.276*	na	na	na
Moist × soilN	-3.02±2.536	-0.002±0.001	na	na	na
Moist × Mic	4.55±2.923	0.111±0.071	na	na	na
soil N × Mic	-0.409±1.226	-0.014±0.042	na	na	na
model r^2	66.3	66.3	57.1	55.2	57.6

[1]Mean coefficients, their SD and significance are estimated using an MCMC 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 (i.e. fixed + random) effects.

Note: In the standardized Microsite interactions model, all sqrt VIFs were <2 except Temperature^2 which was 2.98 and Temperature × Moisture which was 2.30. In the unstandardized Microsite interactions model, all sqrt VIFs were <10 except Temperature which was 16.0 and Temperature^2 which was 14.8.

In the unstandardized Microsite main effects model, all sqrt VIFs were <2 except Temperature and Temperature^2; and the same was observed with the Microclimate model, and the Site-mean climate model.

na = not applicable