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1 **Nitrogen increases early-stage and slows late-stage decomposition**  
2 **across diverse grasslands**

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42  
43 Key words: grasslands, litter decomposition, nitrogen, nitrogen deposition,  
44 Nutrient  
45 Network (NutNet), phosphorus

## Nutrient effects on grassland decomposition

### 46 **Abstract**

47 1. To evaluate how increased anthropogenic nutrient inputs alter carbon  
48 cycling in grasslands, we conducted a litter decomposition study across 20  
49 temperate grasslands on three continents within the Nutrient Network, a  
50 globally distributed nutrient enrichment experiment

51 2. We determined the effects of experimental nitrogen (N), phosphorus (P),  
52 and potassium plus micronutrient ( $K_{\mu}$ ) additions on decomposition of a  
53 common tree leaf litter in a long-term study (maximum of 7 years; exact  
54 deployment period varied across sites). The use of higher-order  
55 decomposition models allowed us to distinguish between effects of nutrients  
56 on early- versus late-stage decomposition.

57 3. Across continents, addition of nitrogen (but not other nutrients)  
58 accelerated early-stage decomposition and slowed late-stage decomposition,  
59 increasing the slowly decomposing fraction by 28% and the overall litter  
60 mean residence time by 58%.

61 4. *Synthesis*. Using a novel, long-term cross-site experiment, we found  
62 widespread evidence that nitrogen enhances the early stages of  
63 aboveground plant litter decomposition across diverse and widespread  
64 temperate grassland sites, but slows late-stage decomposition. These  
65 findings were corroborated by fitting the data to multiple decomposition  
66 models and have implications for nitrogen effects on soil organic matter  
67 formation. For example, following nitrogen enrichment, increased microbial  
68 processing of litter substrates early in decomposition could promote  
69 production and transfer of low molecular weight compounds to soils, and  
70 potentially enhance stabilization of mineral-associated organic matter. By  
71 contrast, by slowing late-stage decomposition, nitrogen enrichment could  
72 promote particulate organic matter (POM) accumulation. Such hypotheses  
73 deserve further testing.

## Nutrient effects on grassland decomposition

- 74 **Keywords:** grasslands, litter decomposition, nitrogen, nitrogen deposition,  
75 Nutrient Network (NutNet), phosphorus

76 **Introduction**

77 Human activities are altering element fluxes at a global scale. For  
78 example, agricultural and industrial activities have greatly increased global  
79 cycling of reactive nitrogen (N) (Ackerman et al. 2019), increasing  
80 atmospheric N inputs relative to phosphorus (P). Yet, we lack a general  
81 mechanistic understanding of how variation in nutrient inputs alters carbon  
82 (C) cycling, hampering the development of Earth System Models to  
83 accurately forecast global C dynamics in a changing world (Peñuelas et al.  
84 2013). Such understanding is especially lacking for grasslands, which  
85 represent ca. 30% of non-agricultural global land area and terrestrial net  
86 primary productivity (NPP) (Chapin et al. 2011) and store 20% of the world's  
87 soil C (FAOSTAT 2009). Effects of N deposition on grassland C cycling will  
88 likely depend on supplies of other nutrients, since NPP in grasslands is often  
89 co-limited by multiple nutrients, with N limitation more prevalent at higher  
90 latitudes (Fay et al. 2015, Cleland et al. 2019, Du et al. 2020). On the other  
91 hand, surface soil C stocks in grasslands can be relatively insensitive to  
92 short-term (ca. 3 years) N and P enrichment, but can increase with short-  
93 term addition of K with micronutrients (Crowther et al. 2019). This  
94 inconsistency between nutrient effects on plant biomass production (NPP),  
95 and thus C inputs to soils, and soil C accumulation indicates a gap in  
96 understanding nutrient effects on grassland litter and soil organic matter  
97 (SOM) decomposition.

98 Past studies of nutrient effects on decomposition in grasslands have  
99 been limited in geographic scope or duration. In studies at individual or  
100 relatively few sites, N had varied effects (Hunt et al. 1988, Aerts et al. 2003,  
101 Liu et al. 2006, Hobbie 2008, Liu et al. 2010, Hou et al. 2021) and P had  
102 neutral effects (Aerts et al. 2003) on decomposition. In a more widespread  
103 but short-term study of tea leaf decomposition (90 days across 21  
104 grasslands), N and P both weakly increased tea leaf decomposition rate and  
105 the tissue stabilization factor, a modeled representation of the biochemically  
106 transformed residues that persist at late stages of decomposition (Ochoa-

## Nutrient effects on grassland decomposition

107 Hueso et al. 2020). Meta-analyses combining experiments done at single or  
108 few sites to generate synthetic insights into nutrient effects on  
109 decomposition have uncovered important among-study patterns (Knorr et al.  
110 2005, Zhang et al. 2018, Gill et al. 2021). However, strong inference from  
111 meta-analysis is limited because it combines data from studies using  
112 different methods (e.g., substrate types, nutrient treatment levels). Most  
113 available studies are of short duration and often lack information on key  
114 covariates (climate, soil chemistry, plant productivity). For example, the  
115 median study duration in a recent meta-analysis of 334 paired  
116 decomposition sequences (from control and associated N-fertilized plots) was  
117 2 years (Gill et al. 2021). In addition, past meta-analyses focused solely on N  
118 (with supply rates varying widely among studies); yet nutrients other than N  
119 might be expected to influence decomposition or interact with N treatments.  
120 While individual studies and meta-analysis provide important insights,  
121 generating a general understanding of nutrient effects on grassland litter  
122 decomposition requires long-term, geographically distributed experiments  
123 that use standard methods and thus overcome the limitations to inference  
124 imposed by the diverse methodologies (e.g., substrate, duration, nutrient  
125 enrichment rates) inherent in meta-analyses (Borer et al. 2014).

126         Long-term studies are needed specifically to determine if nutrients  
127 have different effects early versus later in the decay process. In forests, N  
128 often reduces litter decomposition in its later stages (Fog 1988, Berg and  
129 Matzner 1997, Carreiro et al. 2000, Hobbie et al. 2012, Gill et al. 2021). Such  
130 N inhibition has been attributed to inhibition of oxidative enzyme activity  
131 (Carreiro et al. 2000, Waldrop et al. 2004, Hobbie et al. 2012, Jian et al.  
132 2016, Chen et al. 2018), which may be less strong in grasslands than in  
133 forests, because of their distinct fungal communities with reduced oxidative  
134 enzyme capabilities (Sinsabaugh 2010). On the other hand, if N inhibition of  
135 late-stage decomposition results from other mechanisms, such as decreased  
136 pH and microbial biomass (Treseder 2008), or increased decomposer

## Nutrient effects on grassland decomposition

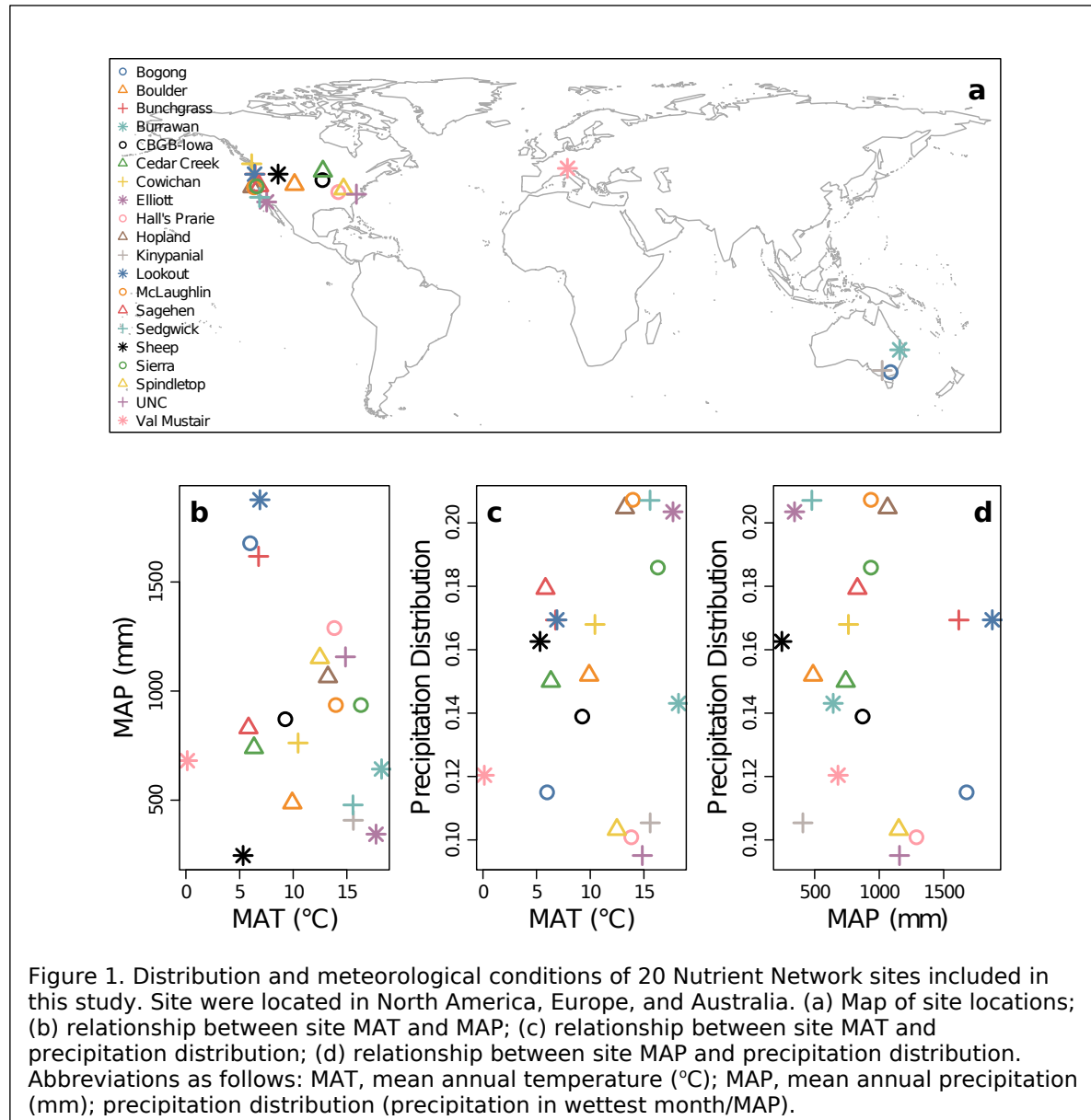
137 necromass accumulation (Gill et al. 2021), such N inhibition might be  
138 expected in grasslands as well as in forests.

139         Cross-site experiments are needed to determine whether effects of  
140 nutrients on litter decomposition are consistent across geographically  
141 diverse sites, or depend on site characteristics such as climate and edaphic  
142 factors. Regional analyses of litter decomposition in grasslands found no  
143 relationships with precipitation or temperature (Bontti et al. 2009, O'Halloran  
144 et al. 2013), although studies at larger spatial scales found evidence for both  
145 (e.g., Tuomi et al. 2009). However, nutrient effects on decomposition might  
146 be more pronounced in high rainfall areas where water does not constrain  
147 microbial activity, as has been shown for net primary production (Burke et al.  
148 1997, Harpole et al. 2007, La Pierre et al. 2016, Ren et al. 2017), or may  
149 depend on ambient soil nutrient supply (Hobbie 2005).

150         Here we decomposed a standard oak leaf litter (to avoid any home-  
151 field advantage effects) for up to seven years in a replicated nutrient  
152 addition experiment in grasslands across multiple continents that ranged  
153 widely in climate and edaphic conditions, to (1) determine the effects of N  
154 and P enrichment on different stages of litter decomposition, and (2) address  
155 whether and how nutrient limitation of leaf litter decomposition varies  
156 geographically. The long duration of the experiment allowed us to determine  
157 which empirical decomposition model structures (single, double, and  
158 asymptotic exponential or Weibull models) best described litter  
159 decomposition dynamics and specifically whether effects of nutrient addition  
160 differed between the early and late stages of decomposition as shown  
161 previously for N, primarily in forests (Gill et al. 2021). Unlike in forests, we  
162 did not expect N to inhibit the later stages of decomposition in these  
163 grasslands because of low oxidative enzyme activity. In addition, we  
164 expected that nutrient effects on decomposition would depend on soil  
165 resource supply, such that N limitation of decomposition would be common  
166 across temperate and alpine regions with young, P-rich soils, but not in areas  
167 with high ambient N deposition or N-rich soils. On the other hand, we

## Nutrient effects on grassland decomposition

168 expected that P limitation would be restricted to sites with highly weathered  
169 soils that are low in available P (e.g., Australia) or to areas with high N  
170 deposition. Furthermore, we hypothesized that nutrient limitation of  
171 decomposition would be strongest in regions with high precipitation or where  
172 moisture supply is relatively constant across the year.



173

## 174 **Materials and Methods**

### 175 *Decomposition Experiment*

176 In the Nutrient Network (NutNet) study, N is added as slow-release  
177 urea ( $10 \text{ g N m}^{-2} \text{ y}^{-1}$ ), P as triple superphosphate ( $10 \text{ g P m}^{-2} \text{ y}^{-1}$ ), and K as



## Nutrient effects on grassland decomposition

178 potassium sulfate ( $10 \text{ g K m}^{-2} \text{ y}^{-1}$ ) with micronutrients (Ca, Mg, S, B, Cu, Fe,  
179 Mn, Mo, and Zn) ( $K_{\mu}$ ) in a full-factorial manner annually to experimental plots.  
180 Twenty sites in the United States, Canada, Australia, and Europe participated  
181 in the present decomposition study (Fig. 1, Table S1), which began <1-2  
182 years following the initiation of fertilization treatments. Sites ranged 17-fold  
183 in atmospheric N deposition ( $1.1 - 18.9 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ), 7-fold in mean annual  
184 precipitation ( $331 - 1898 \text{ mm yr}^{-1}$ ), and 60-fold in mean annual temperature  
185 ( $0.3 - 18.4 \text{ }^{\circ}\text{C}$ ). Precipitation and temperature were unrelated across sites,  
186 allowing us to explore their independent effects. Treatments were applied to  
187  $5 \text{ m} \times 5 \text{ m}$  plots in a randomized block design; for this study, three blocks  
188 were used at all sites except Sierra Foothill, where five blocks were used.

189 We selected leaf litter of *Quercus ellipsoidalis* (Table S2) as a common  
190 substrate for a long-term decomposition study because its N concentration  
191 ( $0.927 \%$ ) fell within the range of N concentrations of grassland leaf litter  
192 (Wedin and Pastor 1993), it was readily abundant and, since it does not  
193 occur in any of the plots, should not exhibit home field advantage effects  
194 that could confound among-site environmental effects. Freshly fallen leaf  
195 litter of *Q. ellipsoidalis* was collected from several adjacent individual trees in  
196 October 2008 at the Cedar Creek Ecosystem Science Reserve, Minnesota,  
197 USA. Litter bags ( $20 \text{ cm} \times 20 \text{ cm}$ , 1-mm mesh fiberglass window screen) were  
198 filled with ca. 10 grams (dry weight) of sterilized leaf litter (autoclaved at  
199  $121^{\circ}\text{C}$  for 15 minutes). Sufficient bags were constructed to allow annual  
200 harvest over seven years and sent to individual site researchers in  
201 September 2009. Bags were strung together in groups of seven and were  
202 pinned to the ground (1 string per plot) using landscape staples. Subsamples  
203 of initial litter were analyzed for (1) C fractions using an ANKOM Fiber  
204 Analyzer (Ankom Technology, Macedon, New York, USA) (cell solubles,  
205 hemicellulose+bound protein, cellulose, and lignin+other recalcitrants)  
206 ( $n=5$ ), (2) initial C and N concentrations on a Costech ECS4010 element  
207 analyzer (Costech Analytical, Valencia, California, USA) ( $n=5$ ), and (3) initial  
208 concentrations of phosphorus (P), magnesium (Mg), calcium (Ca),

## Nutrient effects on grassland decomposition

209 manganese (Mn), and potassium (K) by Inductively Coupled Argon Plasma  
210 Emissions Spectrometry (ICP, Applied Research Laboratory 3560) following  
211 digestion in 10% HCl (Munter and Grande 1981) at the University of  
212 Minnesota's Research Analytical Laboratory (n=1) (Table S2).

213 Sites varied in accessibility and the frequency and timing of research  
214 visits, thus the date of initial deployment ranged from December, 2009 to  
215 October, 2010. Litter bags were sequentially harvested at approximately  
216 annual intervals, and litter was cleaned of any material other than colonizing  
217 microbes, dried (65°C to constant mass), weighed, and sent to the University  
218 of Minnesota for further processing. At the University of Minnesota,  
219 harvested litter was ground, milled, and analyzed for total carbon (C) and N  
220 as above, and a subsample was ashed (600°C for 6 hours) to determine ash-  
221 free dry mass (AFDM). Proportion initial mass remaining was converted to  
222 proportion initial C remaining for further analyses to account for soil  
223 contamination, as soil has low C concentration relative to litter. This method  
224 is similar to correcting for soil contamination using ash-free dry mass, since  
225 they are highly correlated, as confirmed here (across all samples in this  
226 study:  $\%C = 0.75 + 0.50*\%AFDM$ ,  $R^2 = 0.77$ ,  $P < 0.0001$ ). We handled missing  
227 %C data and outlier proportion C remaining values as described in the  
228 Supporting Information.

229 Not all participating sites were able to sample litter bags for the full  
230 duration of the study due to investigator turnover, natural disasters (floods,  
231 herbivory, fires), and unnatural disasters (e.g., loss of sites to development).  
232 In some cases, investigators were unable to visit sites annually. Therefore,  
233 the total number of sites included in the final dataset varied from year to  
234 year (Table S1). Herein we present data from 20 sites that contributed data  
235 from at least three, and up to seven, harvests (Fig. S1).

236 *Temperature, precipitation, solar radiation, and soil physical and chemical*  
237 *properties*

## Nutrient effects on grassland decomposition

238 To understand variation in decomposition across and within sites as  
239 well as interactions between nutrient addition treatments and potential  
240 covariates, we considered modeled site-level predictors, including mean  
241 annual temperature (MAT), mean annual precipitation (MAP), potential  
242 evapotranspiration (PET), aridity index (AI, MAP/PET) (Hijmans et al. 2005),  
243 and precipitation distribution (Precip. Dist., precipitation in wettest  
244 month/MAP; unitless), atmospheric N deposition (Ackerman et al. 2019), and  
245 annual top of atmosphere insolation (NASA GISS  
246 <https://data.giss.nasa.gov/modelE/ar5plots/srlocat.html>; Hansen et al. 2005).  
247 We also considered the following plot-level data: pretreatment soil %C and  
248 %N (determined as above, Crowther et al. 2019), Mehlich 3-extractable P,  
249 Mn, and base cations (K, Ca, Mg, Na) (Waypoint Analytical, Memphis, TN,  
250 USA, Mehlich 1984), and pH (1:1 soil:water suspension, Waypoint Analytical,  
251 Memphis, TN, USA). Aboveground plant biomass was measured by clipping,  
252 drying, and weighing all live and dead plant biomass from two 0.1 x 1 m  
253 strips/plot at peak biomass annually (2010-2016).

### 254 *Overview of Analytical Approach*

255 In our analytical approach, we (1) assessed fit of proportion C mass  
256 remaining to four different decomposition models (single, double, and  
257 asymptotic exponential; Weibull) that differ in how they represent  
258 decomposing litter (e.g., as a single pool decomposing at one rate versus as  
259 multiple pools decomposing at different rates); (2) fit litter N dynamics to a  
260 quadratic function to determine the extent and timing of litter N  
261 immobilization during decomposition; (3) analyzed effects of factorial N x P x  
262  $K_{\mu}$  treatments on parameters obtained from the model-fitting described in (1)  
263 and (2), using treatment-only mixed-effects models, which included N, P, and  
264  $K_{\mu}$  treatments as fixed effects and site and experimental block as nested  
265 random effects; (4) analyzed for potential interactive effects of N, P, and  $K_{\mu}$   
266 fertilization and edaphic and environmental site characteristics on  
267 decomposition model parameters, using treatment-covariate models, which

## Nutrient effects on grassland decomposition

268 included single covariates in mixed-effects models; and (5) used model  
269 selection to identify edaphic and environmental variables that most strongly  
270 explained cross-site variation in decomposition model parameters.

### 271 *Decomposition model fitting*

272 Having up to seven harvests over seven years allowed us to fit litter  
273 proportion C remaining over time to four alternative decomposition models  
274 that vary in mathematical structure, and thus in their representation of the  
275 biophysical process of litter decomposition and chemical composition of the  
276 litter (Olson 1963, Wieder and Lang 1982, Cornwell and Weedon 2014). This  
277 allowed us to explore whether nutrients had different effects in the early  
278 versus later stages of decomposition. The *single exponential decay model*  
279 describes litter as a single pool with a constant decay rate,  $k_s$ , and assumes a  
280 constant fraction of litter mass is decomposed per unit time,  $t$ . The  
281 proportion of initial litter C remaining,  $X$ , is a function of  $k_s$  and  $t$ :

$$282 \quad X = e^{-k_s t} \quad \text{Eqn. 1}$$

284 The *double* and *asymptotic exponential models* divide litter into two pools  
285 that decompose at different rates. In the double exponential model, a labile  
286 fraction,  $L$ , decomposes at rate  $k_1$ , while the remaining slow fraction  $(1-L)$   
287 decomposes at rate  $k_2$ :

$$288 \quad X = L e^{-k_1 t} + (1-L) e^{-k_2 t} \quad \text{Eqn. 2}$$

289 In the asymptotic model,  $A$  describes the slow fraction, which decomposes at  
290 rate zero, while the remaining labile fraction  $(1-A)$  decomposes at rate  $k_a$ :

$$291 \quad X = A + (1-A) e^{-k_a t} \quad \text{Eqn. 3}$$

292 Although the decomposition rate of  $A$  would not realistically be zero,  $A$   
293 approximates a fraction of litter for which the decomposition rate is  
294 negligible (Berg 2000).

295 These first three models represent litter as having one or two discrete  
296 “pools”, each with a constant decomposition rate. A fourth model, the  
297 *Weibull model*, characterizes litter decay as a continuous distribution of

## Nutrient effects on grassland decomposition

298 residence times, as might be expected given the diverse chemistry of  
299 constituents found in litter. This distribution is described by a scale  
300 parameter,  $\beta$ , and a shape parameter,  $\alpha$  (Fréchet 1927, Weibull 1951,  
301 Cornwell and Weedon 2014):

$$302 \quad X = e^{-\left(\frac{t}{\beta}\right)^\alpha} \quad \text{Eqn.}$$

303 4

304 The shape parameter,  $\alpha$ , indicates degree of divergence of decomposition  
305 rates between the early versus later stages:  $\alpha > 1$  indicates that  
306 decomposition is slow early on and then accelerates in the later stages, as  
307 might occur if there is a lag period before decomposition begins, whereas  $\alpha$   
308  $< 1$  indicates more rapid early-stage decomposition relative to the later  
309 stages, as might occur if more labile constituents decompose first, followed  
310 by more complex constituents. When  $\alpha$  equals 1, the Weibull model collapses  
311 to a single-exponential decay model where the instantaneous decay rate is

312 constant and  $k_s = \frac{t}{\beta}$ .

313 As the rate of decomposition described by the Weibull model changes  
314 continuously with time, we cannot compare individual  $k$  values associated  
315 with discrete litter pools. We therefore used the Weibull framework to  
316 calculate the time to 10%, 25%, and 50% mass loss ( $t_{1/10}$ ,  $t_{1/4}$ ,  $t_{1/2}$ , respectively)  
317 and the litter mean residence time ( $MRT$ ) (Cornwell and Weedon 2014) as:

$$318 \quad \text{Eqn. 5}$$

319 and

$$320 \quad MRT = \beta \Gamma\left(1 + \frac{1}{\alpha}\right) \quad \text{Eqn.}$$

321 6

322 where  $p$  is the proportion of litter mass remaining at time  $t$  and  $\Gamma$  is the  
323 gamma function.

324 To determine the most appropriate decomposition models to describe  
325 litter decay dynamics, we initially fit decomposition models to pooled data  
326 from each site x treatment combination (3-5 plots per pool) and compared

## Nutrient effects on grassland decomposition

327 model AIC<sub>c</sub> values to assess the capacity of individual decomposition model  
328 structures to describe decomposition dynamics across treatments (Burnham  
329 and Anderson 2002). Differences of >3 between model AIC<sub>c</sub> values ( $\Delta AIC_c$ )  
330 were used to identify significant differences between model fits. Exponential  
331 models were fit using the *mle2* function in the *bbfme* package (Bolker 2020)  
332 and Weibull models were fit using the *nls* function. To evaluate whether  
333 [nutrient addition changed which model best fit the data](#), we compared which  
334 model was the best fit to the data for all plots that did vs. did not receive N,  
335 P, or K<sub>u</sub>, in three separate contingency analyses.

336 Three of the four decomposition models (single-exponential,  
337 asymptotic, Weibull) best described litter decomposition across sites and  
338 treatments (see Results), so we then fit each of the three models to the  
339 proportion C remaining from all litter bags harvested over time from an  
340 individual plot. To identify outliers within the individual plot models and  
341 obtain more precise parameter estimates for decomposition models, we  
342 calculated the sum of the residual distances between the proportion C  
343 remaining at each litter bag harvest and the predicted value in the Weibull  
344 model, adjusted for the number of predicted values ( $n_{Pred}$ ) in each model  
345 according to the following relationship:

$$346 \quad Model\ Deviance = \frac{\sum [X_{Pred} - X_{Obs}]^2}{n_{Pred}} \quad Eqn.$$

347

7

348 Individual plot models with the highest 2.5% of all deviance scores  
349 were evaluated individually for outliers. In these cases, we iteratively  
350 removed individual data points and re-fit Weibull models to identify predictor  
351 sets that resulted in the lowest model deviance. Individual harvest points  
352 were removed from 22 of 494 decomposition curves and their removal did  
353 not affect analyses presented below. We eliminated entire plots from the  
354 analysis when outlier removal reduced the number of harvest points included  
355 in the model prediction below three (2% of models fit to three points).

## Nutrient effects on grassland decomposition

### 356 *Model fitting to estimate changes in litter N dynamics*

357 Nitrogen is often immobilized initially before being released from  
358 decomposing litter (Staaf and Berg 1981). To describe changes in the litter N  
359 pool throughout the decomposition period, we fit plot-level changes in litter  
360 N pool size through time to quadratic functions with the form:

$$361 \quad Z = (\beta_1 t + \beta_2 t^2) + \beta_0$$

362 Eqn. 8

363 where  $Z$  is the litter N pool (grams),  $t$  is the time since the start of the study  
364 (years),  $\beta_1$  and  $\beta_2$  are coefficients on the  $t$  and  $t^2$  terms, respectively, and  $\beta_0$  is  
365 the initial litter N pool (grams) in each plot. Models were fit to plot-level  
366 decomposition sequences. We used the quadratic function to calculate the  
367 maximum litter N pool ( $N_{max}$ ) and the time to  $N_{max}$  ( $t_{Nmax}$ ) during the litter  
368 decomposition period. We excluded data from individual litter bag harvests  
369 in which the litter C:N ratio fell outside the 95% confidence interval of the  
370 mean for each harvest time point. We also excluded plots with three or fewer  
371 data points describing litter N pool size.

### 372 *Nutrient effects on litter decomposition*

373 We evaluated effects of N, P, and  $K_{\mu}$  addition on parameters describing  
374 early (Weibull  $t_{1/10}$ ,  $t_{1/4}$ , and  $t_{1/2}$ ; asymptotic  $k_a$ ), late (asymptotic  $A$ ), and  
375 overall (single exponential  $k_s$ ; Weibull  $\alpha$ ,  $MRT$ ) litter decomposition using  
376 general mixed models (*nlme* R package) (Pinhiero et al. 2019) in which site  
377 and block were included as nested random effects. These *treatment-only*  
378 statistical models were specified as a full-factorial block design in which N, P,  
379 and  $K_{\mu}$  treatments were included as separate fixed factors. Treatment-only  
380 models were also used to analyze treatment effects on litter N dynamics,  
381  $N_{max}$  and  $t_{Nmax}$ .

382 Using *treatment-covariate* statistical models, we considered whether  
383 inclusion of single covariates – soil pH, soil C (%), atmospheric N deposition  
384 rate ( $\text{kg N ha}^{-1} \text{y}^{-1}$ ), soil P (ppm), soil Mn (ppm), MAT ( $^{\circ}\text{C}$ ), MAP (mm),

## Nutrient effects on grassland decomposition

385 precipitation distribution, annual insolation ( $W\ m^{-2}$ ) or aboveground biomass  
386 ( $g\ m^{-2}$ ) – interacted with nutrient addition treatments or altered the  
387 significance of treatment effects. We fit individual three-way ANCOVA models  
388 that included N, P, and  $K_{\mu}$  fertilization treatments in factorial combination  
389 with each covariate (covariates were considered individually because of the  
390 high number of covariates relative to the number of sites; site and block  
391 were included as nested random effects; *lme* function; *nlme* R package;  
392 Pinhiero et al. 2019). All predictor and response variables were log or square-  
393 root transformed to approximate normality, as appropriate.

### 394 *Cross-site variation in litter decomposition*

395 We used *model selection* to identify predictor variables that best  
396 described cross-site variation in decomposition model parameters (Weibull  $\alpha$ ,  
397 *MRT*,  $t_{1/10}$ ,  $t_{1/4}$ , and  $t_{1/2}$ ; asymptotic  $k_a$ ,  $A$ ; single-exponential  $k_s$ ). We considered  
398 predictors describing climate and edaphic conditions, including MAT, MAP, AI,  
399 PET, and precipitation distribution; soil C, N, P, Mn, and base cation content;  
400 soil pH; and modeled N deposition rate. After screening potential predictors  
401 for collinearity (Pearson  $R > |0.70|$ , Table S3; *Hmisc* R package; Harrell  
402 2020), we excluded AI, which was highly correlated with MAT; PET, which  
403 was highly correlated with MAT and AI; soil N, which was highly correlated  
404 with soil C; the sum of the extractable base cations, which was highly  
405 correlated with pH; and annual insolation, which was highly correlated with  
406 MAT (Pearson's  $R > 0.70$ ). The final set of predictors is presented in Table  
407 S3.

408 We fit individual global mixed-effects models with all potential  
409 predictors (MAT, MAP, Precip. Dist., soil C, soil P, soil Mn, soil pH, annual N  
410 deposition rate, and aboveground plant biomass; Table S3) for each  
411 response variable using the *lme* function in the *nlme* R package (Pinhiero et  
412 al. 2019). We then generated a full submodel set from the global model  
413 using the 'dredge' function and considered all two-way interactions (*MuMIn* R  
414 package) (Nakagawa and Schielzeth 2013). McLaughlin Natural Reserve and



## Nutrient effects on grassland decomposition

415 Hopland Research and Extension Center in California, USA, did not report  
416 initial soil chemistry, and thus sites were excluded from the model selection  
417 analysis, while Val Mustair in Europe was excluded as modeled N deposition  
418 data were unavailable. Experimental block nested within site was included as  
419 a random effect in each model, but fertilization treatments were excluded  
420 from models.

421 We present the top models with  $\Delta AIC < 3$  and conditional model  
422 average associated with each parameter. For individual candidate models  
423 with  $\Delta AIC < 3$ , we calculated the variance explained by the fixed effects in  
424 isolation (marginal  $R^2$ ) and that explained by both the fixed and random  
425 effects in combination (conditional  $R^2$ ; *MuMIn* R package) (Nakagawa and  
426 Schielzeth 2013). All analyses were conducted using R version 3.5.3 (R  
427 Foundation for Statistical Computing, Vienna, Austria, 2018).

### 428 **Results**

429 Across all sites and treatments, the median percentage C remaining at  
430 the end of the experiment was 36%. Sites with the fastest decomposition  
431 were Cowichan, British Columbia; Spindletop Farm, Kentucky; University of  
432 North Carolina; and Hall's Prairie, Kentucky (with 4, 7, 12, and 14% initial C  
433 remaining on average across treatments, respectively). Sites with the  
434 slowest decomposition were Sheep Experiment Station, Idaho; Kinypanial,  
435 Victoria; and Sagehen Creek Field Station, California (with 55, 59, and 62%  
436 initial C remaining, respectively). Formal comparisons of mass loss among  
437 treatments and sites were not possible because of different duration of litter  
438 deployment across sites. Hereafter, we focus on analyses of parameters  
439 obtained from fitting decomposition models.

#### 440 *Decomposition Model Fits*

441 Using  $AIC_c$  was to compare model fits, within the exponential decay  
442 model family, single and asymptotic decomposition models provided the best  
443 fits to the data, whereas the double exponential model was a poor fit to the  
444 data (Table S4.A). Considering the Weibull decomposition model along with

## Nutrient effects on grassland decomposition

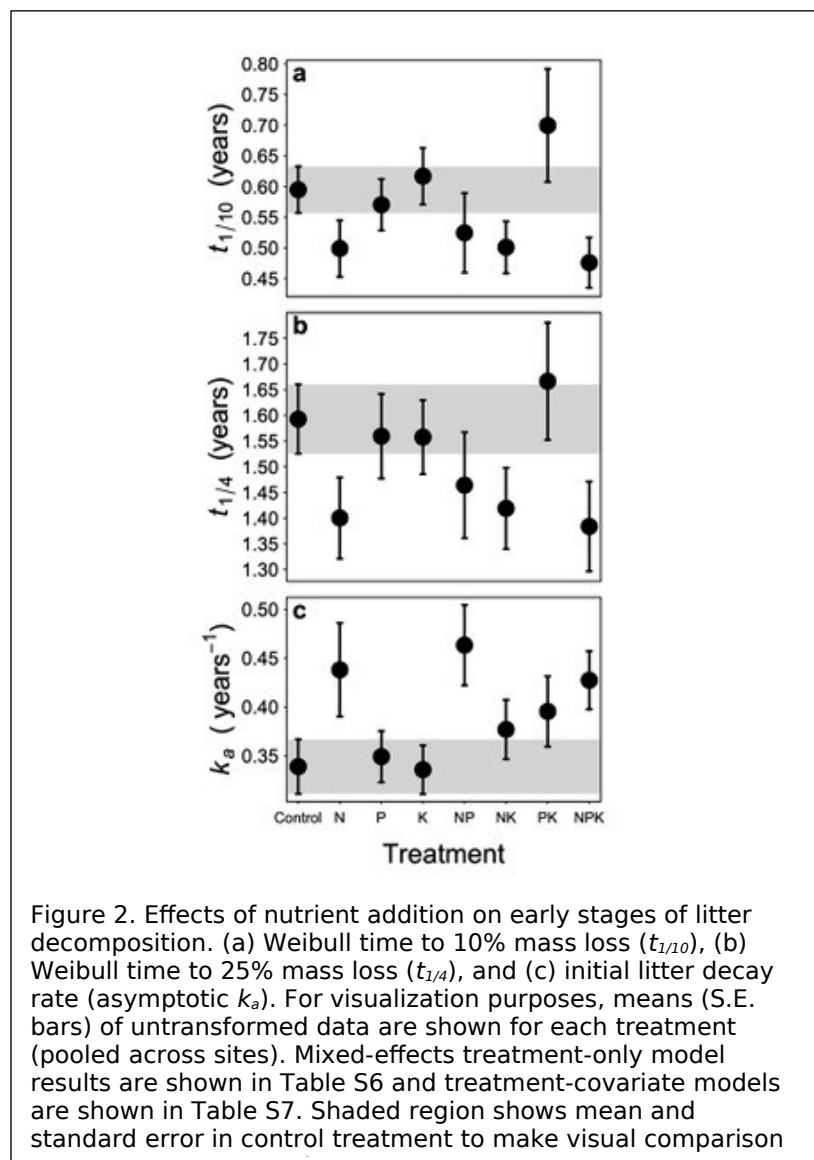
445 the exponential decay models, the Weibull model fit the data as well as the  
446 best exponential model fit in 61% of all *site\*treatment* combinations (Table  
447 S4.B). Because AIC<sub>c</sub> was consistently higher for the double exponential  
448 model than the other models, we therefore fit the single, asymptotic, and  
449 Weibull models to all individual plots.

450 While neither N, P, nor K<sub>μ</sub> changed which decomposition model best fit  
451 the data (Table S5.A), by contrast, the best model to describe litter  
452 decomposition differed across sites (Table S5.B). The single exponential  
453 model best described the majority of the treatments at 13 sites, the  
454 asymptotic model at three sites, and the Weibull model at one site. At two  
455 sites, the single exponential and Weibull models were the best fit for an  
456 equal number of treatments. The double exponential was never the best  
457 model for any treatments at any sites.

458 *Effects of experimental nutrient addition on early stage litter decay ( $t_{1/10}$ ,  $t_{1/4}$ ,  
459  $t_{1/2}$ ,  $k_a$ )*

460 In treatment-only statistical models, N addition (but not P or K<sub>μ</sub>) greatly  
461 accelerated early-stage decomposition, decreasing the Weibull time to 10%  
462 and 25% mass loss ( $t_{1/10}$  and  $t_{1/4}$ ) and increasing the initial decay rate  
463 (asymptotic  $k_a$ ), by 16%, 12%, and 23% respectively (Fig. 2, Table S6).  
464 Similarly, in treatment-covariate models, N fertilization (but neither P nor K<sub>μ</sub>)  
465 reduced  $t_{1/10}$  and  $t_{1/4}$  and increased asymptotic  $k_a$  (Table S7). However, in  
466 contrast to our expectations, N-induced increases in early-stage litter  
467 decomposition were not stronger in sites with high P or base cation  
468 availability. There were some weak interactive effects of fertilization and  
469 other site factors on early-stage decomposition (Table S7), with N more  
470 strongly increasing early-stage decomposition ( $k_a$ ) in sites with low soil Mn  
471 content (Fig. S1) and pH (Fig. S2). K<sub>μ</sub> increased  $t_{1/10}$  and  $t_{1/4}$  in sites with more  
472 evenly distributed precipitation (not shown), while P and K<sub>μ</sub> increased  $t_{1/2}$  and  
473 decreased  $t_{1/4}$ , respectively, in sites with high pH (Fig. S2).

## Nutrient effects on grassland decomposition



474

475 *Effects of nutrient addition on late stage and overall litter decay ( $\alpha$ , MRT,  $k_s$ ,*

476 *A)*

477 In contrast to the early stages of litter decomposition, in treatment-  
 478 only models, there were no significant main effects of any nutrients on the  
 479 single exponential decomposition rate ( $k_s$ ) (Fig. 3; Table S6). However, both N  
 480 and P, but not K, increased the Weibull MRT, and N decreased Weibull  $\alpha$  and  
 481 increased the fraction of slowly decomposing C (asymptotic A). Similarly, in  
 482 treatment-covariate models, N increased Weibull MRT and A (Table S7),  
 483 indicating that N caused rates to slow as decomposition proceeded.  
 484 However, N effects on early and late-stage decomposition were largely

## Nutrient effects on grassland decomposition

485 offsetting, as N had only a small effect on the single-exponential decay  
486 model ( $k_s$ ), increasing it by 6% on average (Fig. 3, Fig. 4, Table S7). The  
487 decrease in Weibull  $\alpha$  with added N, from values  $> 1$  to values closer to 1,  
488 indicated that N accelerated early-stage decomposition (Fig. 4). Phosphorus  
489 also generally increased Weibull  $MRT$  and sometimes increased the fraction  
490 of slowly decomposing C ( $A$ ), indicating it also tended to slow the later stages  
491 of decomposition. Phosphorus also affected litter half-life ( $t_{1/2}$ ) in models that  
492 included covariates, but complex N x P x  $K_\mu$  interactions made those effects  
493 difficult to interpret (Table S7).

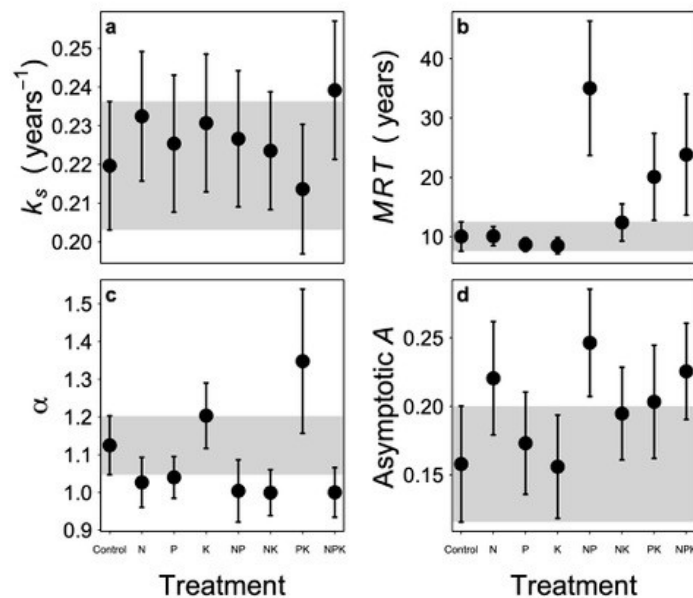


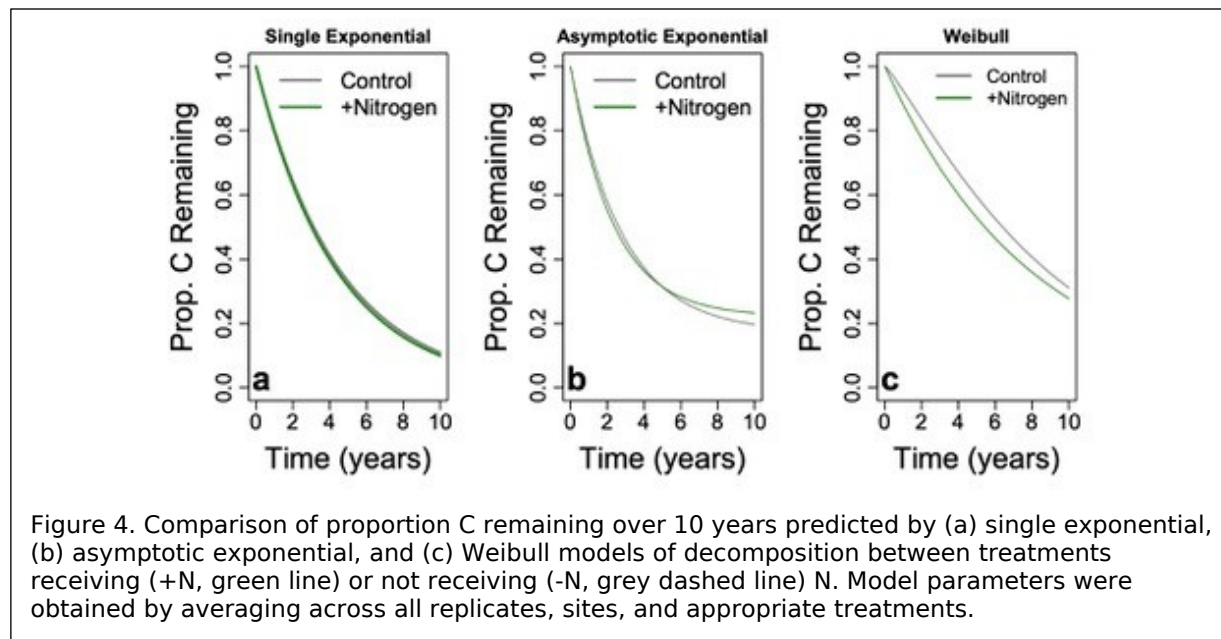
Figure 3. Effects of nutrient addition on decomposition parameters describing the entire time course of decomposition, (a) single exponential  $k_s$ , (b) Weibull  $MRT$ , and (c) Weibull  $\alpha$ ; or later stages of decomposition, (d) asymptotic  $A$ . For visualization purposes, means (S.E. bars) of untransformed data are shown for each treatment (pooled across sites). Mixed-effects treatment-only model results are shown in Table S6 and treatment-covariate models are shown in Table S7. Shaded region shows mean and standard error in control treatment to make visual comparison among treatments easier.

494

495 Fertilization interacted with several site factors in its effects on late-  
496 stage and overall decomposition dynamics (Table S7). N increased single  
497 exponential  $k_s$  more at sites with low than high N deposition (albeit weakly,  
498 Fig. S3). Nitrogen also increased  $k_s$  in sites with low Mn (Fig. S1), but reduced  
499  $k_s$  and Weibull  $\alpha$ , and increased Weibull  $t_{1/2}$  and  $MRT$ , and asymptotic  $A$  more  
500 where precipitation was more evenly distributed across the year (low Precip.

## Nutrient effects on grassland decomposition

501 Dist; Fig. S4). Finally, N reduced Weibull  $\alpha$  and  $k_s$  and increased Weibull  $MRT$   
502 more at sites with higher aboveground biomass (Fig. S5).



503  
504 Nitrogen was typically immobilized in leaf litter in the initial stages of  
505 decomposition. Plots with faster early-stage decomposition (higher  
506 asymptotic  $k_a$ , lower Weibull  $t_{1/10}$ ) exhibited higher maximum litter N pools  
507 ( $N_{max}$ , Table S8). Higher  $N_{max}$  was also associated with increased litter  $MRT$   
508 and asymptotic  $A$ , as well as reduced Weibull  $\alpha$ . In treatment-only models, N  
509 fertilization increased litter  $N_{max}$  (N:  $p < 0.0001$ ), as well as the time to the  
510 maximum litter N content ( $p = 0.031$ , Table S9, Fig. S6).

### 511 *Variables predicting litter decay dynamics across sites*

512 In the model selection analysis, climatic and edaphic factors were  
513 unable to explain substantial variation in decomposition model parameters  
514 describing the early stages of decay (Weibull  $t_{1/10}$ ,  $t_{1/4}$ ; asymptotic  $k_a$ ; Table  
515 S10). Less than 10% of the cross-site variation in these parameters was  
516 captured by fixed effects, indicating that most of the variation among sites in  
517 the initial decomposition rates was caused by unmeasured site factors (Table  
518 S10).

519 By contrast, MAT, background N deposition, and precipitation variation  
520 were modest to strong predictors of Weibull half-life ( $t_{1/2}$ ), parameters

## Nutrient effects on grassland decomposition

521 describing later-stage decomposition (asymptotic  $A$ ), or parameters  
522 describing the entire decomposition curve (Weibull  $\alpha$ ,  $MRT$ ; single  
523 exponential  $k_s$ ) (Table S10). Generally, warmer sites had faster  
524 decomposition (lower  $t_{1/2}$ , lower Weibull  $MRT$ ), sites with more variable  
525 seasonal precipitation (higher Precip. Dist.) had slower decomposition  
526 (longer Weibull  $MRT$ , lower  $k_s$ ), and sites with higher levels of atmospheric N  
527 deposition had faster decomposition (lower Weibull  $MRT$ , lower asymptotic  $A$ ,  
528 Tables S7, S10).

### 529 **Discussion**

#### 530 *Contrasting effects of N on early versus late-stage decomposition*

531 In a long-term (seven-year) experiment replicated across grasslands  
532 spanning diverse biotic, edaphic, and climatic conditions, N consistently  
533 accelerated early-stage decomposition. This points to N limitation of  
534 microbes decomposing the N-poor *Quercus* litter, whereby added N  
535 increased decomposer C demand. Neither P nor  $K_{\mu}$ , alone or in combination  
536 with N, limited decomposition in its early stages, in contrast to nutrient co-  
537 limitation of NPP in grasslands (Fay et al. 2015, Cleland et al. 2019, Du et al.  
538 2020). Decomposer N demand likely was met via fungal translocation of soil  
539 N in control plots (Frey et al. 2000, Chigineva et al. 2011, Xiong et al. 2014),  
540 but via fertilizer-supplied N in N-treated plots. Accordingly, litter in the N-  
541 fertilized plots exhibited less initial delay of decomposition and greater N  
542 immobilization than litter in control plots. Our findings corroborate  
543 observations that across diverse litter types, low-N litter types exhibited  
544 lagged decomposition dynamics relative to high-N litter (Cornwell and  
545 Weedon 2014). In addition to alleviating N limitation, N may have affected  
546 early-stage decomposition via other mechanisms. A study in semi-arid  
547 grassland attributed positive effects of N on decomposition to acidification  
548 that increased Mn availability and altered the microbial community (Hou et  
549 al. 2021). However, while N fertilization also reduced soil pH (at 10/20 sites)  
550 in the present study, the magnitude of pH reduction was unrelated to the

## Nutrient effects on grassland decomposition

551 effects of N on decomposition model parameters (data and analyses not  
552 shown).

553 Our findings corroborate past studies demonstrating widespread N  
554 stimulation of early-stage decomposition, and are the first to show this using  
555 a long-term experiment in diverse grasslands. A short-term study of  
556 decomposing tea leaves across 21 Nutrient Network sites also found positive  
557 N effects on early-stage decomposition (Ochoa-Hueso et al. 2020).

558 Phosphorus addition also positively affected early-stage decomposition in  
559 that study perhaps because the relatively high N concentrations in tea  
560 increased microbial P demand. In a meta-analysis of N fertilization effects on  
561 decomposition in diverse ecosystems, N fertilization reduced  $t_{1/10}$  and  $t_{1/4}$  and  
562 increased Weibull  $MRT$  and asymptotic  $k_a$ , as found here (Gill et al. 2021).

563 Our findings are also consistent with results from some local to regional  
564 cross-site studies, predominantly in forests, showing N stimulation of initial  
565 decomposition (Berg and Matzner 1997, Hobbie et al. 2012).

566 In contrast to our hypothesis, N inhibited later-stage decomposition  
567 (increasing asymptotic  $A$  and increasing Weibull  $MRT$ ), as found in many  
568 forest studies (Berg and Matzner 1997, Whittinghill et al. 2012, Gill et al.  
569 2021), and in a short-term grassland study, where N increased the Tea Bag  
570 Index stabilization factor (a modeled representation of the biochemically  
571 transformed tea leaves that persist at late stages of decomposition) (Ochoa-  
572 Hueso et al. 2020). Inhibition of late-stage decomposition by added N has  
573 been attributed to several interrelated mechanisms including suppression of  
574 oxidative enzyme activity; formation of recalcitrant compounds from  
575 accumulated microbial necromass or reactions of inorganic N with litter  
576 metabolites; Mn limitation; and suppression of microbial biomass by  
577 acidification (Fog 1988, Berg and Matzner 1997, Carreiro et al. 2000,  
578 Treseder 2008, Zak et al. 2008, Hobbie et al. 2012, Entwistle et al. 2018,  
579 Morrison et al. 2018, Whalen et al. 2018, Gill et al. 2021). The concordance  
580 of results presented here with those from forest and short-term grassland

## Nutrient effects on grassland decomposition

581 studies points to the generality that elevated N slows late-stage  
582 decomposition in terrestrial ecosystems.

583 We hypothesized that N would not inhibit late-stage decomposition in  
584 grasslands because of relatively low plant lignin content, and associated low  
585 basidiomycete abundance and lignin-degrading oxidative enzyme activity  
586 (Sinsabaugh 2010). Indeed, meta-analyses published well after our study  
587 began show little evidence that N inhibits oxidative enzyme activity in  
588 grasslands (Jian et al. 2016, Chen et al. 2018, Xiao et al. 2018). However,  
589 depressive effects of N enrichment on grassland microbial biomass (Ramirez  
590 et al. 2012) could have slowed decomposition.

591 It is also possible that alleviation of microbial N limitation early in  
592 decomposition led to necromass accumulation on litter surfaces and  
593 contributed to the apparent reduction in late-stage litter because of  
594 necromass constituents that decompose more slowly than the plant  
595 compounds consumed by those decomposers (Liang et al. 2019, Gill et al.  
596 2021). Increased N immobilization in response to N fertilization, which was  
597 associated with reduced rates of late-stage decomposition, provides indirect  
598 support for this mechanism. Concurrent measures of enzyme activity and  
599 decomposer necromass are needed to distinguish among these potential  
600 mechanisms.

601 Contrasting N effects early and late in decomposition, as found here,  
602 have implications for N effects on SOM. Early in decomposition, N could  
603 promote litter processing and transfer of low molecular weight compounds to  
604 soils, potentially enhancing stabilization of mineral-associated organic matter  
605 (MAOM). By contrast, larger slowly decomposing litter fractions could  
606 promote accumulation of particulate organic matter (POM) and total SOM  
607 (Cotrufo et al. 2015). Interestingly, a study conducted in nine North American  
608 NutNet sites found no effects of N on MAOM or POM (Keller et al.  
609 unpublished), raising uncertainty about whether differential effects of N over  
610 the course of litter decomposition have consistent consequences for SOM.



## Nutrient effects on grassland decomposition

611 *Utility of higher-order models in describing litter decomposition dynamics*

612 Multi-parameter empirical models illuminated divergent N effects early  
613 vs. late in decomposition. Thus model structure choice influences  
614 interpretation of litter decomposition dynamics, with implications for  
615 experimental design and hypothesis testing (Cornwell and Weedon 2014).  
616 Single-parameter models readily fit data from experiments with fewer  
617 collection points, but may mischaracterize decomposition dynamics that shift  
618 through time. Herein, the single exponential model often provided equivalent  
619 AIC<sub>c</sub> fit to two- or three-parameter models, suggesting that the single  
620 exponential model sufficiently described the complexity of grassland litter  
621 decomposition. However, the Weibull model structure fit the data equally  
622 well or better than the single exponential model for 54% of site-treatment  
623 combinations, and the asymptotic model fit the data equally well or better  
624 for 83%. As the fertilization responses observed here and in other recent  
625 syntheses (Gill *et al.*, 2021) emerged only when models allowed  
626 decomposition rates to change through time, these higher-order model  
627 structures deserve consideration in both experimental design and data  
628 analysis.

629 Whether N addition would differently affect *Quercus* litter  
630 decomposition relative to native litter at each site due to variation in litter  
631 chemistry, lack of home-field advantage effects (Palozzi and Lindo 2018), or  
632 sterilization prior to deployment, is unclear. In a study across eight locations  
633 at one of our study sites (Cedar Creek), where the litter used here originated,  
634 litter of *Quercus ellipsoidalis* and *Schizachyrium scoparium*, a dominant C4  
635 grasses in Cedar Creek grasslands and savannas, had similar initial acid-  
636 nonhydrolyzable fractions (lignin and other recalcitrants) and N  
637 concentrations, similar rates of decomposition, and responded  
638 idiosyncratically to added N in grasslands in the early and late stages of  
639 decomposition (Hobbie 2005, 2008). However, the *Quercus* litter used here  
640 had higher concentrations of lignin (Table S2) than would be expected for  
641 herbaceous species on average (e.g., Oliveira *et al.* 2020), which could have

## Nutrient effects on grassland decomposition

642 accentuated negative effects of N on the later stages of decomposition if the  
643 underlying mechanism was related to N inhibition of oxidative enzyme  
644 activity.

### 645 *Interactions between nutrient treatment effects and site factors*

646 We found limited support for hypothesized interactions between site  
647 factors and nutrient treatments on decomposition, suggesting that N effects  
648 (and lack of P and K<sub>μ</sub> effects) were general across diverse sites. We had  
649 expected that nutrients would limit decomposition more in warm, wet  
650 regions, yet N limitation of early-stage decomposition was largely  
651 independent of climate. However, negative effects of N on late-stage  
652 decomposition were strongest in sites where precipitation was more evenly  
653 distributed across the year. As the frequency of wetting events regulates  
654 nutrient diffusion to litter bags, more consistent precipitation regimes may  
655 support elevated N environments throughout the year, contributing to  
656 elevated microbial biomass and higher necromass stabilization. Negative N  
657 effects also increased with aboveground biomass, which may protect litter  
658 from UV radiation and prevent decomposer desiccation, supporting increased  
659 necromass stabilization.

660 Across 20 sites with widely varying geography, results did not support  
661 our hypothesis of greater N limitation of decomposition in younger, less  
662 weathered sites, and greater P limitation in older more weathered sites.  
663 Instead, N increased early-stage decomposition regardless of soil chemistry  
664 and climate, and the only consistent P effect was to increase Weibull *MRT*.

### 665 *Variation in decomposition across sites*

666 Mean annual temperature, precipitation variability, and atmospheric N  
667 deposition all contributed to variation in decomposition rates across sites,  
668 mostly later in decomposition. Faster decomposition in warmer sites,  
669 observed previously in global decomposition syntheses (Gholz et al. 2000,  
670 Zhang et al. 2008, Kwon et al. 2021), is expected because of greater  
671 biological activity, longer growing seasons, and higher nutrient availability.

## Nutrient effects on grassland decomposition

672 Greater photodegradation in warmer sites could also have contributed to faster  
673 decomposition in those sites, given that MAT was highly correlated with  
674 annual insolation. Faster decomposition in sites where precipitation was  
675 distributed more evenly relative to annual precipitation likely reflects the  
676 importance of moisture in limiting decomposition in grasslands, especially at  
677 times of year when temperatures are favorable (Meentemeyer 1978, Gholz  
678 et al. 2000). Indeed, a manipulation of rainfall variability showed that lower  
679 precipitation variability, resulting in higher average soil moisture, led to more  
680 rapid decomposition (Walter et al. 2013).

681 Positive relationships between  $k_s$  and N deposition provide further  
682 evidence that N limits decomposition across grasslands, and contrast results  
683 from a cross-site study of tea leaf decomposition worldwide, which showed  
684 negative relationships between atmospheric N deposition and mass loss over  
685 3 and 12 months in temperate sites (Kwon et al. 2021). Effects of N from  
686 deposition and fertilizer differed in key ways. First, whereas fertilizer N  
687 reduced the initial lag period of slow decomposition, atmospheric N  
688 deposition was associated with a longer lag period. This discrepancy could  
689 have arisen if long-term effects of chronic N addition were not yet apparent  
690 in fertilized plots, which began receiving fertilizer <1 to 2 years before litter  
691 bags were deployed. For example, N deposition might have altered microbial  
692 communities, as seen in long-term (> 8 y) fertilization studies (Frey et al.  
693 2004, Hesse et al. 2015, Entwistle et al. 2018, Whalen et al. 2018, Hou et al.  
694 2021), in ways that slowed colonization of litter by decomposer organisms  
695 and reduced litter-soil hyphal connections. Other effects of chronic N  
696 deposition associated with soil acidification (e.g., reduced base cation  
697 supply) (Hou et al. 2021) might also have manifested slowly in N fertilization  
698 treatments.

699 While sites with higher N deposition had smaller litter slow pools, N  
700 fertilization increased the slow pool. As discussed previously, numerous long-  
701 term decomposition studies have demonstrated that N fertilization increases  
702 the slow pool (asymptotic  $A$ ). Fewer studies evaluated relationships of  $A$  with

## Nutrient effects on grassland decomposition

703 ambient N deposition rates. Rates of N addition in fertilization studies,  
704 including ours, are often higher than the highest rates of N deposition in  
705 polluted regions. Herein, N was added at a rate of  $100 \text{ kg N ha}^{-1} \text{ y}^{-1}$ , whereas  
706 the highest rate of N deposition across sites was ca.  $20 \text{ kg N ha}^{-1} \text{ y}^{-1}$ .  
707 Although the specific mechanism by which added N increased the slow pool  
708 is unknown, perhaps its effects only occur at relatively high rates of added N.

### 709 *Unexplained site variation*

710 Initial decomposition rates varied widely among sites, but in ways not  
711 explained by measured site climate or edaphic factors. Across six grassland  
712 sites in the US Central Great Plains, decomposition of common aboveground  
713 substrates was similarly unrelated to climate factors (Bontti et al. 2009) and  
714 a cross-sites study of decomposing tea leaves similarly found a high  
715 percentage of unexplained variation across grassland sites (Kwon et al.  
716 2021). What other factors might have contributed to variation in initial  
717 decomposition across sites? Variation in the decomposer community (Prober  
718 et al. 2015) and how quickly it colonized the litter could have contributed to  
719 site variation in initial decomposition because of variation in metabolic  
720 capabilities of microbial decomposers (Strickland et al. 2009, Gessner et al.  
721 2010, Bray et al. 2012, Glassman et al. 2018). Other factors, such as the  
722 contributions of soil fauna to decomposition or vascular plant community  
723 composition, might also have contributed to site-to-site variation in  
724 decomposition (García-Palacios et al. 2013). For example, the site with the  
725 highest total decomposition (Cowichan) is dominated by densely growing  
726 rhizomatous grasses, whose roots completely encased the litter bags after  
727 several years. Previous decomposition trials at this site also observed rapid  
728 biomass decomposition (Ziter and MacDougall 2013). Photodegradation is  
729 also important for aboveground decomposition in grasslands and could have  
730 contributed to site variation in decomposition dynamics (Austin and Vivanco  
731 2006, Adair et al. 2017), although greater aboveground biomass was  
732 associated with faster  $k_s$  in control plots (but not in plots receiving N),

733 opposite what might be expected if photodegradation contributed  
734 substantially to initial decomposition rates. The fiberglass mesh used in this  
735 study transmits about 45-50% of solar radiation (Adair et al. 2017), so  
736 photodegradation could have contributed to mass loss. We were unable to  
737 evaluate the effects of PAR at the ground surface on decomposition across  
738 sites because of lack of data, so we cannot rule this out as a potential  
739 mechanism. Nevertheless, variation in irradiance, soil contamination of the  
740 litter bags (Brandt et al. 2010, Adair et al. 2017), and position of the bags  
741 relative to the ground surface, standing, and fallen vegetation (Lin and King  
742 2014) could have caused variation in photodegradation and biotic  
743 decomposition.

#### 744 **Concluding Remarks**

745         In a long-term (seven-year) study of decomposition of a common leaf  
746 litter substrate in a factorial fertilization experiment replicated at 20  
747 grassland sites spanning a wide range of biotic, edaphic, and climatic  
748 conditions, N fertilization consistently increased early-stage and slowed late-  
749 stage decomposition, as often seen in forests. These patterns were  
750 discernable because of the long duration of the study, the large number of  
751 sites, and the use of decomposition models that could distinguish early- and  
752 late-state decomposition dynamics. These findings corroborate those of Gill  
753 et al. (2021) in showing that fitting decomposition data to single exponential  
754 models can obscure effects of environmental factors on decomposition  
755 dynamics. Implications of contrasting early- versus late-stage effects of N on  
756 litter decomposition for SOM pools and dynamics deserve further empirical  
757 study and exploration using ecosystem models.

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## Nutrient effects on grassland decomposition

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### 772 **Author Contribution**

773 SEH and ALG contributed equally. ETB, EWS, WSH, SEH, and KSH designed  
774 the research. ETB, CRB, EEC, CMD, KFD, DSG, WSH, SEH, ASM, RLM, BAM,  
775 JLM, JWM, ACR, MS, EWS, JW, and LHY performed the research. PBA, ETB,  
776 CRB, EEC, CMD, KFD, ALG, DSG, WSH, SEH, ASM, RLM, BAM, JLM, JWM, ACR,  
777 MS, EWS, JW, and LHY contributed to data analysis, collection or  
778 interpretation. PBA, ETB, EEC, CMD, KFD, ALG, WSH, SEH, KSH, ASM, RLM,  
779 ACR, MS, EWS, JW, and LHY contributed to writing the manuscript.

### 780 **Data Availability**

781 Data are archived and available: [https://www.cedarcreek.umn.edu/research/  
782 data/dataset?aige247](https://www.cedarcreek.umn.edu/research/data/dataset?aige247),  
783 doi:10.6073/pasta/2ebd7eaf2fac27e1c7eee9678baa7940. Code to replicate  
784 analyses and figures are available:  
785 <https://github.com/gill20a/NutNetLitterDecomp>.

### 786 **Figure Legends**

787 Figure 1. Distribution and meteorological conditions of 20 Nutrient Network  
788 sites included in this study. Site were located in North America, Europe, and  
789 Australia. (a) Map of site locations; (b) relationship between site MAT and  
790 MAP; (c) relationship between site MAT and precipitation distribution; (d)  
791 relationship between site MAP and precipitation distribution. Abbreviations as

## Nutrient effects on grassland decomposition

792 follows: MAT, mean annual temperature (°C); MAP, mean annual precipitation  
793 (mm); precipitation distribution (precipitation in wettest month/MAP).

794

795 Figure 2. Effects of nutrient addition on early stages of litter decomposition.  
796 (a) Weibull time to 10% mass loss ( $t_{1/10}$ ), (b) Weibull time to 25% mass loss  
797 ( $t_{1/4}$ ), and (c) initial litter decay rate (asymptotic  $k_a$ ). For visualization  
798 purposes, means (S.E. bars) of untransformed data are shown for each  
799 treatment (pooled across sites). Mixed-effects treatment-only model results  
800 are shown in Table S6 and treatment-covariate models are shown in Table  
801 S7. Shaded region shows mean and standard error in control treatment to  
802 make visual comparison among treatments easier.

803 Figure 3. Effects of nutrient addition on decomposition parameters describing  
804 the entire time course of decomposition, (a) single exponential  $k_s$ , (b) Weibull  
805  $MRT$ , and (c) Weibull  $\alpha$ ; or later stages of decomposition, (d) asymptotic  $A$ .  
806 For visualization purposes, means (S.E. bars) of untransformed data are  
807 shown for each treatment (pooled across sites). Mixed-effects treatment-only  
808 model results are shown in Table S6 and treatment-covariate models are  
809 shown in Table S7. Shaded region shows mean and standard error in control  
810 treatment to make visual comparison among treatments easier.

811 Figure 4. Comparison of proportion C remaining over 10 years predicted by  
812 (a) single exponential, (b) asymptotic exponential, and (c) Weibull models of  
813 decomposition between treatments receiving (+N, green line) or not  
814 receiving (-N, grey dashed line) N. Model parameters were obtained by  
815 averaging across all replicates, sites, and appropriate treatments.

816

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