

1 Decomposition disentangled: a test of the multiple mechanisms by which nitrogen enrichment alters  
2 litter decomposition

3

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26 Species richness

## 27 Summary

28

29 1. Nitrogen (N) enrichment has direct effects on ecosystem functioning by altering soil abiotic  
30 conditions and indirect effects by reducing plant diversity and shifting plant functional  
31 composition from dominance by slow to fast growing species. Litter decomposition is a key  
32 ecosystem function and is affected by N enrichment either by a change in litter quality (the  
33 recalcitrance of the plant material) or through a change in soil quality (the abiotic and biotic  
34 components of the soil that affect decomposition). The relative importance of soil and litter  
35 quality and how the direct and effects of N alter them remains poorly known.

36 2. We designed a large grassland field experiment manipulating N enrichment, plant species  
37 richness and functional composition in a full factorial design. We used three complementary  
38 litter bag experiments and a novel structural equation modelling approach to quantify the  
39 relative effects of the treatments on litter and soil quality and their importance for total  
40 decomposition.

41 3. Our results indicate that total decomposition was mostly driven by changes in litter quality  
42 rather than soil quality. Litter quality was affected by the nutrient contents (N and calcium)  
43 and structural components of the litter (leaf dry matter content, fibres). N enrichment  
44 increased litter decomposition mostly indirectly through a shift in functional composition  
45 toward faster growing plant species producing higher quality litter. N enrichment also had  
46 effects on soil, by directly and indirectly affected vegetation cover, but this had relatively few  
47 consequences for the total decomposition rate.

48 4. *Synthesis*. Our approach provides a mechanistic tool to test the drivers of litter decomposition  
49 across different ecosystems. Our results show that functional composition is more important  
50 than richness or soil quality in determining litter decomposition and that N enrichment effects  
51 mainly occur via above- rather than belowground processes. This highlights the importance

52 of considering shifts in plant species composition when assessing the effects of N enrichment  
53 on decomposition.

## 54 Introduction

55

56           Soil nitrogen enrichment is one of the major global changes ecosystems are currently facing  
57 (Galloway et al., 2008). Nitrogen (N) enrichment alters ecosystem functioning directly and through  
58 several indirect mechanisms. It directly alters functions related to nutrient stocks and fluxes by  
59 changing soil abiotic conditions, stoichiometry and pH (Sardans, Rivas-Ubach, & Peñuelas, 2012;  
60 Laliberté & Tylianakis, 2012). In addition N enrichment indirectly affects ecosystem functioning by  
61 altering biotic community properties such as plant diversity and composition. N enrichment typically  
62 reduces the number of plant species able to coexist (Suding et al., 2005) and this loss of diversity could  
63 affect ecosystem functioning as much as N addition per se. (Hooper et al., 2012; Tilman, Reich, & Isbell,  
64 2012). However, plant community change, following N enrichment, does not only involve a loss of  
65 species it also involves compositional turnover and in particular a shift towards faster growing plant  
66 species (Isbell et al., 2013; Lavorel & Grigulis, 2012; de Vries et al., 2012). This shift is indicated by an  
67 increase in mean values of trait linked to the leaf economics spectrum, such as specific leaf area and  
68 leaf N content, (Wright et al., 2004) and this shift is a key driver of ecosystem functioning (Lavorel &  
69 Grigulis, 2012). However, we still have little mechanistic insight into the relative importance of these  
70 direct (abiotic) and indirect (plant richness and composition) effects of N enrichment on ecosystem  
71 functioning. Observational studies have separated direct effects of N from indirect effects mediated  
72 through species richness (Isbell et al., 2013) and/or functional composition (Allan et al., 2015).  
73 However, observational studies struggle to separate effects of correlated drivers, such as diversity loss  
74 and compositional turnover. Experimental approaches are therefore needed to separate these effects  
75 and to fully understand and predict the mechanisms by which N addition affects ecosystem  
76 functioning.

77           The decomposition of plant litter is a key ecosystem function that influences rates of soil  
78 biogeochemical cycling and which is strongly affected by N deposition (Finn et al., 2015; Knorr, Frey,  
79 & Curtis, 2005; Hobbie et al., 2012). Depending on the ecosystem, the enrichment level and duration,

80 N can have either positive or negative effects on decomposition (Bardgett & Wardle, 2012; Knorr et  
81 al., 2005; Hobbie et al., 2012; Riggs, Hobbie, Bach, Hofmockel, & Kazanski, 2015) and to understand  
82 this variation we need to better understand the mechanisms behind them. Plant litter decomposition  
83 is determined by multiple mechanisms: it depends principally on the physical and chemical properties  
84 of the litter and on soil biotic and abiotic conditions (Cebrian, 1999; Handa et al., 2014; Cornwell et  
85 al., 2008). To distinguish these two main drivers of litter decomposition, we will refer to “litter quality”,  
86 as the physical and chemical properties of litter that affect its decomposition and to “soil quality”, as  
87 the soil biotic and abiotic factors which determine decomposition rates. Both soil and litter quality are  
88 key determinants of litter decomposition but their relative importance, especially following N  
89 enrichment, is not well known (but see Cleveland et al., 2014; García-Palacios, Prieto, Ourcival, &  
90 Hättenschwiler, 2016b; Maaroufi, Nordin, Palmqvist, & Gundale, 2017). N enrichment could influence  
91 decomposition by directly or indirectly changing both soil quality (i.e. by altering soil properties and  
92 fauna), and litter quality. To understand the impacts of N enrichment on decomposition we need  
93 experimental and analytical approaches that can separate these different, cascading mechanisms.

94 N enrichment is likely to directly and indirectly alter litter quality and therefore decomposition  
95 rates. Litter quality is largely determined by its chemical properties (nutrient contents and the  
96 presence of defence compounds) and by physical factors such as leaf dry matter and fibre contents  
97 (Garnier et al., 2004; Cornwell et al., 2008). With higher soil N availability, plants will produce more  
98 rapidly degradable tissues with higher N contents and fewer fibres. In addition to N, macronutrients  
99 like Ca and Mg may also influence litter decomposability (García-Palacios, McKie, Handa, Frainer, &  
100 Hättenschwiler, 2016a) and their availability could also be altered by N addition (Aber et al., 1998).  
101 Indirect effects of N are also likely to be important: a shift to fast growing plant communities further  
102 enhances litter quality because fast growing plants have generally higher leaf N and lower fibre  
103 contents. Fast growing plants also invest less in defences against herbivores and pathogens  
104 (Blumenthal, Mitchell, Pysek, & Jarosík, 2009) and have fewer chemicals such as tannins that reduce  
105 decomposition. However, other indirect effects of N may reduce decomposition. A reduction in

106 species and functional diversity could reduce decomposability (Handa et al., 2014). Although some  
107 aspects of litter quality are well characterised, we lack a comprehensive picture of how N enrichment  
108 alters these different aspects simultaneously.

109         Enriching soils with N is likely to alter their quality for litter decomposition both directly and  
110 indirectly. The abundance and composition of the soil macro, meso and microfauna are key  
111 determinants of soil quality (Milcu & Manning, 2011) as macrofauna fragment large litter pieces,  
112 which accelerates decomposition by smaller organisms (Milcu, Partsch, Scherber, Weisser, & Scheu,  
113 2008). N enrichment could increase soil quality if it causes a shift towards bacterial dominated  
114 communities (from fungal dominated ones), either through direct effects of N or through changes in  
115 plant functional composition, which is likely to lead to increased decomposition rates (Fierer,  
116 Strickland, Liptzin, Bradford, & Cleveland, 2009; Bardgett & McAlister, 1999; Bardgett & Wardle, 2012;  
117 de Vries, Hoffland, van Eekeren, Brussaard, & Bloem, 2006). However, N enrichment might indirectly  
118 reduce soil quality if a loss of plant diversity loss results in a loss of soil organism diversity (Milcu et al.,  
119 2013). In addition, N addition will directly increase plant biomass (in N limited systems), but might  
120 indirectly reduce it by reducing diversity (van der Plas, 2019; Isbell et al., 2013), and a change in  
121 biomass will alter microclimatic conditions such as soil temperature and moisture, which are  
122 important drivers of decomposition (Hättenschwiler, Tiunov, & Scheu, 2005; Blankinship, Niklaus, &  
123 Hungate, 2011). The various direct and indirect effects of N enrichment are therefore likely to have  
124 complex and potentially opposing effects on soil quality and therefore on litter decomposition rates.

125         In this study, we tested the effects of N enrichment on litter decomposition and disentangled  
126 its direct effects on soil and litter quality from its indirect effects mediated by plant richness and  
127 functional composition. We created experimental plant communities to realise a full factorial cross of  
128 plant functional composition, plant species richness and N enrichment. Plant functional composition  
129 was manipulated by creating a gradient in community mean specific leaf area and leaf N as these traits  
130 are key indicators of resource economics and plant growth strategy. Three complementary litter bag

131 experiments were used to test direct and indirect effects of N addition on litter quality, on soil quality  
132 and on both combined. We also looked at the effect of macro and mesofauna on decomposition by  
133 using different mesh sized litter bags. This framework enabled us to test the following questions:

134 What is the relative importance of direct effects of N enrichment on decomposition relative to indirect  
135 effects mediated through changes in the plant community (species richness and functional  
136 composition)?

137 Is decomposition determined more by changes in litter quality or soil quality?

138 How important are meso and macro fauna in determining decomposition and how does their relative  
139 importance change with N enrichment?

140

## 141 Material and methods

142

### 143 The PaNDiv Experiment

144

145 The PaNDiv Experiment is located in Münchenbuchsee near the city of Bern (Switzerland,  
146 47°03'N, 7°46'E, 564 m.a.s.l.). It has a mean annual temperature of  $9.2 \pm 0.61^\circ\text{C}$  and mean annual  
147 precipitation of  $1051.78 \pm 168.42 \text{ mm y}^{-1}$  (mean over the last 30 years, data from the Federal Office of  
148 Meteorology and Climatology MeteoSwiss). The soil is characterized as 0.7 to 1m deep brown earth  
149 (Cambisol), according to the Geoportal of the Canton Bern (<http://www.geo.apps.be.ch>). We  
150 measured total soil N and carbon (C) concentrations and pH in the top 20 cm of soil at the start of the  
151 experiment and found concentrations of 2.3-4.2% C, 0.26-0.43% N and a pH of 7.4. The field site had  
152 been extensively managed (without fertilization) for at least 10 years before the start of the

153 experiment and had been used for fodder production and grazing. The vegetation was cleared and the  
154 area ploughed before the experimental plots were established.

155         The species sown were selected from a pool of 20 species commonly found in both extensively  
156 and intensively managed Central European grasslands. We divided our 20 species into 10 fast and 10  
157 slow growing species according to their Specific Leaf Area (SLA) and leaf N content, which are related  
158 to resource use strategy (see Figures S1 and S2) (Wright et al., 2004). The fast growing pool therefore  
159 corresponds to species found in N enriched sites, whereas the slow growing pool comprises species  
160 found in less productive sites. We excluded legumes from the species pool as few legume species will  
161 grow well at high N levels and including legumes only in the slow growing pool would have caused an  
162 additional and large difference between the species pools. We realised several combinations of fast  
163 and slow growing species, so effects of changes in mean traits are independent of particular species  
164 effects.

165         In order to separate direct and indirect effects of N enrichment, we established a factorial  
166 cross of treatments representing the direct (N enrichment) and indirect effects (plant diversity loss  
167 and change in functional composition) on 2x2m plots. Fertilised plots received N in the form of urea  
168 twice a year in April and late June (beginning of the growing season and following the first cut, see  
169 below), for an annual addition of 100 kg N ha<sup>-1</sup>y<sup>-1</sup>, which corresponds to intermediately intensive  
170 grassland management (Blüthgen et al., 2012). To manipulate diversity, we established plots with 1,  
171 4, 8 or all 20 species. To manipulate functional composition and diversity we established plots with  
172 only fast growing, only slow growing or a mix of fast and slow growing species. This allowed us to  
173 realise a large gradient in community weighted mean trait values, which is crossed with functional  
174 diversity, as mixed plots have higher diversity than single strategy plots. Functional composition,  
175 functional diversity and species richness were all completely crossed at the 4 and 8 species levels  
176 (monocultures and 20 species plots could only contain one functional composition). We sowed all  
177 plants in monoculture and we established four replicates of the 20 species together. At the four and



178 eight species levels we randomly selected species compositions: we selected 10 species compositions  
179 for each combination of richness (4 and 8), times functional composition (fast, slow mixed). This meant  
180 we had a total of 20 monocultures, 30 four species compositions, 30 eight species compositions and  
181 four replicates of 20 species composition. We constrained the random selection to ensure that all  
182 polycultures contained both grasses and herbs. The 84 different species compositions were grown  
183 once in control conditions and once with N addition. In addition to the N treatment, we also applied  
184 a fungicide treatment and a fungicide x N treatment, resulting in 336 plots in total. However, for  
185 logistical reasons the litter bag experiment was only conducted on the 168 control (no fungicide) plots  
186 (see Table S1). The whole field was divided into four blocks. Each block contained all 84 compositions  
187 but the particular N x fungicide treatment was randomly allocated per block. A regularly mown 1m  
188 path sown with a grass seed mixture consisting of *Lolium perenne* and *Poa pratensis* (UFA-  
189 Regeneration Highspeed) separated the plots.

190 All species within a plot were sown at equal density in October 2015, with proportions corrected by  
191 species specific germination rates, to obtain a total density as close as possible to 1000 seedlings m<sup>-2</sup>.  
192 The seeds were obtained from commercial suppliers (UFA Samen, Switzerland, and Rieger-Hofmann,  
193 Germany). Some species were resown once in spring 2016 because of poor establishment (*Heracleum*  
194 *sphondylium*, *Anthriscus sylvestris*, *Daucus carota*, *Salvia pratensis*, *Prunella grandiflora*, *Plantago*  
195 *media*), because they were mixed with other seeds to begin with (*Helictotrichon pubescens*, *Bromus*  
196 *erectus*) or because their seedlings froze in autumn or spring (*Holcus lanatus*, *Dactylis glomerata*,  
197 *Anthoxanthum odoratum*). No resowing was done after spring 2016. In order to maintain the diversity  
198 levels, the plots were weeded three times a year in April, July and September. This regime was highly  
199 successful and most plots contained very low weed covers in the following season (Figure S3). The  
200 whole experiment was mown twice a year in mid-June and mid-August which corresponds to  
201 intermediate to extensive grassland management.

202

## 203 Measuring decomposition of litter bags

204

205 We conducted three complementary litter bag experiments simultaneously to test the  
206 mechanisms by which our treatments affected decomposition. The first set of bags tested the effect  
207 of our treatments on the soil quality. We filled those bags with rapeseed straw (*Brassica napus*) as a  
208 standard material and placed them on every plot. No Brassicaceae are present in the experiment and  
209 this litter should therefore be equally foreign for all plots. To test the effect of our treatments on litter  
210 quality (decomposability), we filled a second set of bags with biomass collected from each plot and let  
211 them decompose in a common garden, established in the grassland surrounding the experimental  
212 plots. We filled the third set of bags, called plot bags, with aboveground dry biomass from each plot  
213 and let them decompose on their own plot (i.e. the plot from which the biomass was collected) to test  
214 the combined effect of soil and litter quality on decomposition. By combining data from these three  
215 experiments, we can disentangle the relative importance of soil and litter quality in driving overall  
216 decomposition.

217 We sewed the litterbags using nylon fabric with a mesh size of 5 mm for the above part and  
218 0.2 mm for the fabric in contact with the soil, to avoid loss of material during transport and  
219 manipulation (Bradford, Tordoff, Eggers, Jones, & Newington, 2002). To investigate the effects of  
220 different sized groups of detritivores on decomposition, we sewed two additional plot bags: a 2 mm  
221 mesh size to exclude the macrofauna, and a 0.2 mm mesh size to exclude meso and macrofauna (Milcu  
222 & Manning, 2011; Bardgett, 2005). By comparing decomposition rates in the bags with different mesh  
223 sizes we can estimate the effect of different aspects of the soil community on the overall  
224 decomposition rate.

225 The plant biomass used to fill the common garden and plot bags was collected on the field  
226 before the mowing in June 2017 (with some very unproductive plots sampled again in August in order  
227 to have enough material). Green litter differs in its composition from senescent litter due to nutrient

228 resorption (Aerts, 1996), and therefore decomposes at a different rate (Sanaullah, Chabbi, Lemaire,  
229 Charrier, & Rumpel, 2010). We were, however, more interested in the difference in decomposition  
230 among plant communities rather than in measuring the absolute decomposition rate. In addition,  
231 green litter decomposition is an important process in grasslands which are managed by cutting and  
232 many similar decomposition experiments have therefore also used green litter (Sanaullah et al., 2010;  
233 Vogel, Eisenhauer, Weigelt, & Scherer-Lorenzen, 2013). The biomass was dried at 65°C for 48h,  
234 chopped, homogenized and split into equal parts (Biomass splitter, RT 6.5–RT 7; Retsch, Haan,  
235 Germany). We filled each bag with a maximum of 20g dry material and weighed the litterbags again  
236 after closing. Because some experimental communities produced only a small amount of biomass, we  
237 could not include 20g in all bags and the initial biomass varied from 5 to 20g. The bags decomposed  
238 on top of the soil for 2.5 months between September and December 2017. We then collected the  
239 bags, cleaned them of debris and soil, dried them and weighed them again. We measured  
240 decomposition rate as the percentage biomass lost between September and December, to correct for  
241 differences in initial weight. Initial bag weight was included as a covariate in our models but it never  
242 affected the percentage mass loss (see Table S3).

243

## 244 Plant traits used to calculate functional composition

245

246 To produce a continuous measure of functional composition for all our plant communities we  
247 calculated community weighted means for Specific Leaf Area (SLA) and Leaf Dry Matter Content  
248 (LDMC). Although plots were designed to differ in SLA, we also created a large gradient in mean LDMC,  
249 which was only partially correlated with SLA. We measured SLA and LDMC in the control (unfertilised)  
250 monocultures and therefore did not include any plasticity in response to N addition, in order to ensure  
251 that the community weighted mean traits were as orthogonal to N addition as possible. We sampled  
252 one leaf from five individuals per species and followed the protocol of Garnier, Shipley, Roumet, and

253 Laurent (2001) and measured the fresh weight and leaf area with a leaf area meter (LI-3000C, LI-COR  
254 Biosciences) after a minimum of 6h and a maximum of 2 days of rehydration in the dark. We dried the  
255 samples at 65°C for two days and measured their dry weight. To measure the abundances of the plant  
256 species, we visually estimated the percentage cover of our target and weed species on every plot  
257 before the biomass was cut. In total three people estimated cover but there was no systematic  
258 difference in the species relative covers estimated by the three recorders (data not shown). We  
259 calculated a Community Weighted Mean (CWM) trait measure for each plot by multiplying each  
260 species' relative abundance (cover) by the mean trait value of the species in monoculture ( $CWM = \sum$   
261  $p_i * x_i$ ; with  $p_i$  the relative abundance of the species  $i$  and  $x_i$  the trait value of  $i$ ).

262

## 263 Litter quality

264

265 Two key aspects of litter quality are nutrient and fibre contents. We measured the  
266 concentration of several nutrients and fibre fractions in the plant biomass. We analysed biomass  
267 samples of all plots from June and August 2017 using Near Infrared Reflectance Spectrometry (NIRS).  
268 A minimum of 5 g of biomass per plot (pooled sample, including all species present and their relative  
269 abundance) was ground with a cyclone mill to obtain a fine powder. The infrared spectrum of the  
270 powder was used to estimate the nutrient and fibre contents based on calibration models developed  
271 for aboveground grassland biomass by Kleinebecker, Klaus, and Hölzel (2011). We estimated acid  
272 detergent fiber (ADF: cellulose, lignin and silica), neutral detergent fiber (NDF: ADF + hemicellulose)  
273 and acid detergent lignin (ADL: crude lignin fraction) in the biomass, as well as concentrations of N, C,  
274 phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg).

275 We could not use all nutrients and fibre fractions separately in the analyses as some of them were  
276 highly correlated (e.g. Mg and Ca, see Figures S4 and S5). We decided to select a widely used set of  
277 variables that did not correlate strongly and which together account for structural components and

278 nutritional quality of litter: biomass N, fibres (ADF) and Ca content (García-Palacios et al., 2016a; Smith  
279 & Bradford, 2003; Cornwell et al., 2008). We did not include ratios like C:N or lignin:N as they were  
280 closely correlated with other variables and did not add more information to the model.

281 In addition to our measures of functional composition (CWMs) and mean values of litter quality, we  
282 calculated a measure of litter quality diversity. For this we used the abundance weighted Mean  
283 Pairwise Distance metric (MPD) (de Bello, Carmona, Lepš, Szava-Kovats, & Pärtel, 2016). This measure  
284 quantified the distance between all species in a plot in their SLA, LDMC, biomass N, fibre and Ca values.  
285 In order to derive species specific values for biomass N, Ca and fibres, we used the values from the  
286 control monocultures as the species trait values, as for SLA and LDMC.

287

## 288 **Analyses**

289

290 We first used linear mixed effect models to test the effect of our treatments on litter  
291 decomposition (percentage mass loss), for each bag individually and for all sets of bags combined. We  
292 ran two combined models: one with plot litter, standard litter and common garden litter combined  
293 and one with the three mesh sizes combined. We ran the models in R (package lme4, Bates, Mächler,  
294 Bolker, & Walker, 2015; R Core Team, 2018) and simplified full models by dropping terms that did not  
295 significantly improve the overall model fit, using likelihood-ratios. All models included block and  
296 species composition (84 levels) as random terms. Species composition distinguished the randomly  
297 assembled sets of species and was included to correct for the fact that replicated species composition  
298 are pseudoreplicates for testing the species richness effects. The combined model with all the bags  
299 also included plot as a random term (168 levels). We added fixed covariates for the month of biomass  
300 harvest (June or August) and the initial weight of biomass put in each bag. We did not transform the  
301 data since the errors were normally distributed and the variance homogenous.

302 The first type of models tested the effects of the treatments on each bag:

303           % mass loss ~ Nitrogen \* Species richness \* Functional composition + Harvest date + Initial  
304           weight + (1|Block) + (1|Combination)

305 The second type of models tested for interactions between bag type (plot, standard, common garden  
306 litter; or the three mesh sizes) and the treatments:

307           Common garden, Standard and Plot bags

308           % mass loss ~ Nitrogen \* Species richness \* Functional composition \* Bag type + Harvest date  
309           + Initial weight + (1|Block) + (1|Combination) + (1|Plot number)

310 Although we used categorical measures of functional composition to design the experiment, we  
311 intended to create a gradient in CWM traits. We therefore replaced our three level functional  
312 composition variable by a continuous measure of community weighted mean SLA and LDMC, and  
313 functional diversity (MPD). For instance, in a single model:

314           % mass loss ~ Nitrogen \* Species richness \* (SLA + LDMC + MPD) + Harvest date + Initial weight  
315           + (1|Block) + (1|Combination)

316

317           In a second step, we quantified the mechanisms by which our treatments affected decomposition  
318 using Structural Equation Modelling (SEM) (Grace, 2006). We included our three decomposition  
319 experiments (and the different mesh size treatments, see below) in the same model. By doing this we  
320 were able to test, not only the effect of our treatments on litter or soil mediated decomposition, but  
321 also the relative importance of litter and soil mediated decomposition for driving the final  
322 decomposition rate measured per plot. We used the mass loss in the "plot" litter bags (i.e. litter  
323 decomposing on its own plot) as a measure of the total plot decomposition rate. We then used the  
324 mass loss in the common garden litter bags as a measure of the litter mediated effects on  
325 decomposition, as these bags decompose on the same soil and only variation in litter quality will

326 determine variation in mass loss between the bags. We used mass loss from the standard litter bags  
327 as our measure of soil mediated decomposition rates. In these bags the litter is always the same and  
328 therefore only variation in soil quality between plots will determine variation in decomposition. We  
329 fitted paths from common garden and standard litter mass loss to plot litter mass loss. The size of  
330 these two standardised path coefficients indicates the relative contribution of litter and soil quality to  
331 overall decomposition rates. In the SEM, plot litter mass loss is only affected by the mass loss  
332 measured in common garden and standard litter bags, to determine if we can explain all of the  
333 variation in overall decomposition rate based on our two measures of litter and soil quality.

334 We then tried to identify the traits and community properties that determined litter and soil  
335 quality. To do this we included our manipulated variables, N addition and plant species richness, as  
336 well as continuous measures of plant functional composition and litter quality, SLA, LDMC, biomass N,  
337 fibres and Ca, in the SEM. These measures could affect functional diversity (MPD) and microclimate.  
338 The microclimate measure we used in the analyses is the total plant cover on each plot. It correlates  
339 with biomass production and accounts for humidity and temperature variation among plots (Figure  
340 S6). To account for an effect of the soil fauna on decomposition, we included the log response ratio of  
341 the big mesh to the small mesh bag decomposition rate (see Figure S7). This variable "soil fauna effect"  
342 measures the relative effect of macro and mesofauna exclusion on decomposition and tests whether  
343 our treatments alter their effect.

344 We fitted SEMs using the lavaan package (Rosseel, 2012). This meant we could not include random  
345 effects, which could bias paths from species richness to other variables (which are not corrected for  
346 species composition). However, we also fitted models using piecewiseSEM (Lefcheck, 2016), in which  
347 we could include composition as a random effect, and this did not change the significance of any paths  
348 (see Table S2). Our initial model was rejected. We therefore included four residual covariance terms  
349 suggested by the lavaan modification indices. Including these covariances substantially improved  
350 model fit and led to a well supported model, however, it did not change the significance or

351 substantially alter the strength of any paths. The first additions were negative covariances between  
352 biomass N and MPD and between LDMC and MPD. These are justified because monocultures (coded  
353 as zero MPD) had a greater range in biomass N and LDMC measures than polycultures, meaning some  
354 monocultures had much higher biomass N content than any of the polycultures. The two other  
355 covariances were between soil fauna effect and plot decomposition, and between litter quality and  
356 plot decomposition. These covariances are reasonable because we used the same litter in these  
357 different bags and a residual covariance is therefore likely. The residual covariance between plot and  
358 litter quality was fitted alongside a directed path and indicated the influence of unmeasured variables  
359 on both terms. The theoretical model and all detailed hypothesis are described in the Supplementary  
360 Information (Figure S7).

361

## 362 Results

363

### 364 Individual effects of N enrichment and plant community characteristics on litter and soil 365 quality

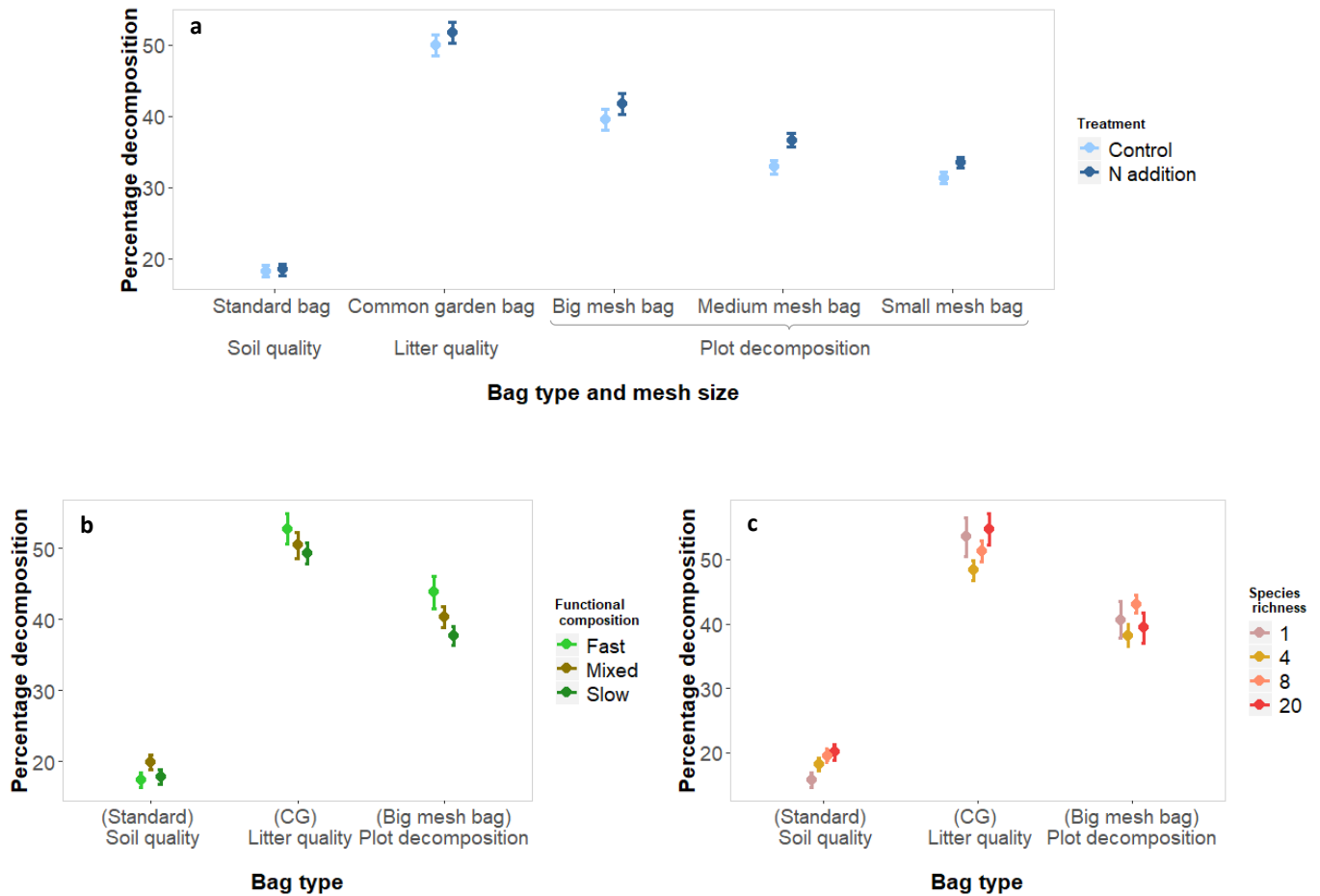
366

367 Decomposition rates differed significantly among bag types. Litter decomposed faster in the  
368 common garden than on the experimental plots, and standard litter decomposed most slowly.  
369 Decomposition rates increased with mesh size (Fig.1a and Table S3).

370 N enrichment increased the litter decomposition rate in all bags consistently (significant main effect  
371 of N but no interaction between N and mesh size, Table S3 and Figure 1a). The effect was absent for  
372 standard litter bags when analysed alone but was significant when different bag types (common  
373 garden, standard, and plot big mesh size bags) were analysed together. There was no interaction



374 between N and mesh size, meaning that N enrichment did not change the relative effect of large fauna,  
 375 compared to small fauna, on decomposition.

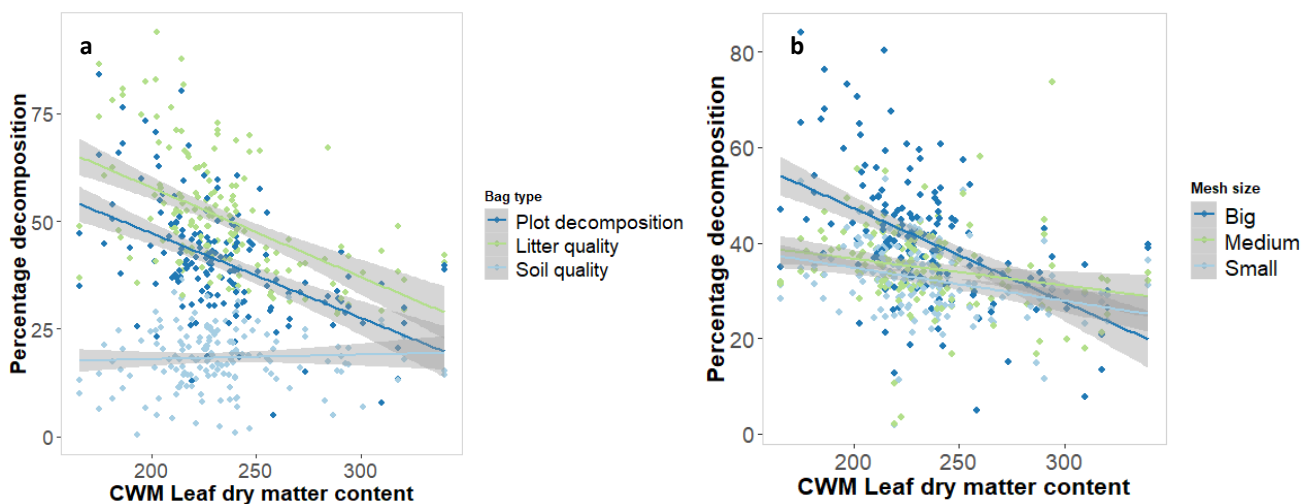


**Figure 1.** Effect of nitrogen addition (a), functional composition (b) and species richness (c) on litter decomposition depending on the litter bag type (standard, common garden and plot decomposition) and the mesh size (big, medium and small). Mean and standard error of the raw values (168 plots per bag).

376

377 Plant functional composition, expressed as a categorical variable (fast, mixed or slow growing species,  
 378 Fig.1b), had a significant effect on the decomposition of common garden and plot litter. Litter from  
 379 fast growing communities decomposed more rapidly than litter from mixed and slow communities.  
 380 We observed the same pattern with continuous measures of functional composition, with a non-  
 381 significant effect of SLA but a negative significant effect of LDMC on decomposition (Figure 2). LDMC  
 382 therefore seemed to be a better predictor than SLA of the effect of growth strategy on decomposition.

383 Comparing the bags with different mesh sizes, LDMC had a larger negative effect on decomposition in  
384 the big mesh litter bags than in the smaller mesh sizes, suggesting a larger effect of LDMC on the  
385 activity of the macrofauna than on the activity of the meso or microfauna (Fig 2b).  
386 Plant species richness had a positive effect on the decomposition of standard litter bags, when  
387 analysed separately (Fig.1c and Table S3). The effect of functional diversity depended on the bag type,  
388 with a negative effect in plot and common garden bags and a positive effect on standard bags (Table  
389 S3). These results indicate that species richness and functional diversity of communities increased soil  
390 quality, whereas the functional composition of the community increased litter quality.



**Figure 2.** Effect of community weighted mean leaf dry matter content ( $\text{mg g}^{-1}$ ) on decomposition depending on the bag type (a) and on the mesh size (b). Mean and standard error of the raw values (168 plots per bag).

391

392

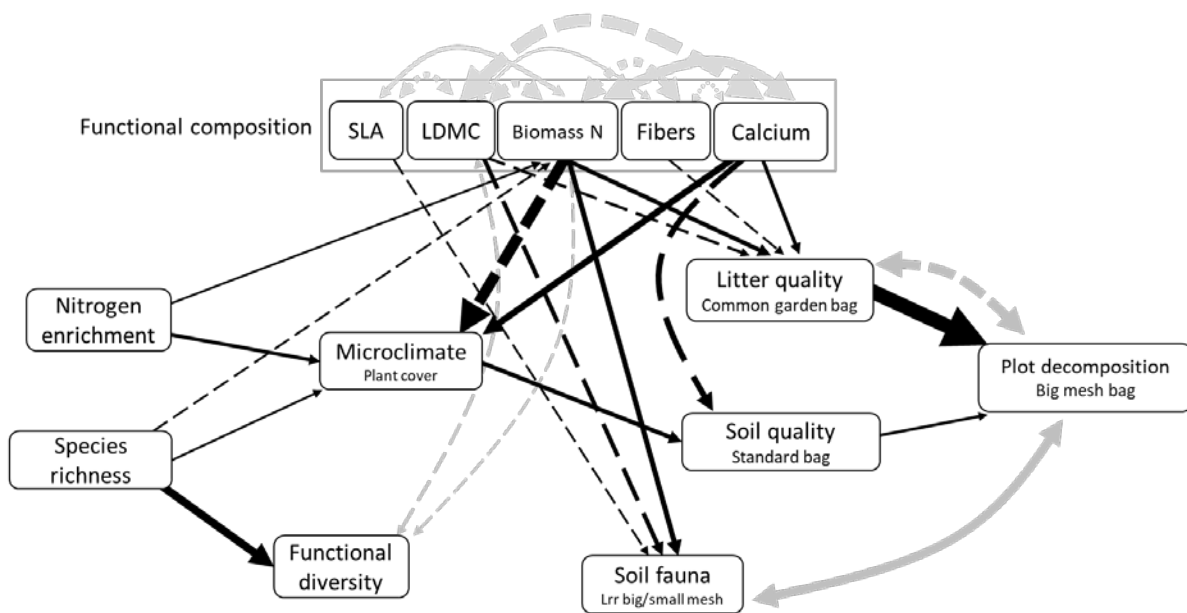
### 393 Relative importance of litter and soil quality in driving overall decomposition

394

395 We used structural equation models to test the relative importance of our different  
396 treatments in affecting soil and litter quality and the relative importance of litter and soil in driving  
397 the overall decomposition rate. Litter and soil quality both had a positive effect on total plot

398 decomposition, but litter quality was much more important (path coefficient of 0.96, Table S4) than  
399 soil quality (path coefficient of 0.20; see Figures 3 and 4a and b). Although soil macro and mesofauna  
400 increased decomposition overall, they did not contribute to variation in decomposition between plots,  
401 as there was no link between the log response ratio between decomposition in big and small mesh-  
402 sized bags and the overall decomposition rates.

403



**Figure 3.** Final results of the structural equation model, showing effects of nitrogen enrichment, plant species richness and plant functional composition on decomposition. Dashed arrows show negative, full arrows positive path coefficients. The arrow size is proportional to the path coefficient. Double-headed grey arrows show covariances. Details of the output in Table S4. Model fit: Pvalue 0.423; Chisq 17.477; Df 17; RMSEA 0.013.

404

405 Litter quality was mainly influenced by plant functional composition. Litter from communities with a  
406 high biomass N content, low LDMC and low fibre content, corresponding to our fast growing  
407 communities, decomposed faster than litter from slow growing communities (Fig. 4e-h). Interestingly,  
408 high Ca concentrations in the biomass also increased litter quality (path coefficient of 0.32). In

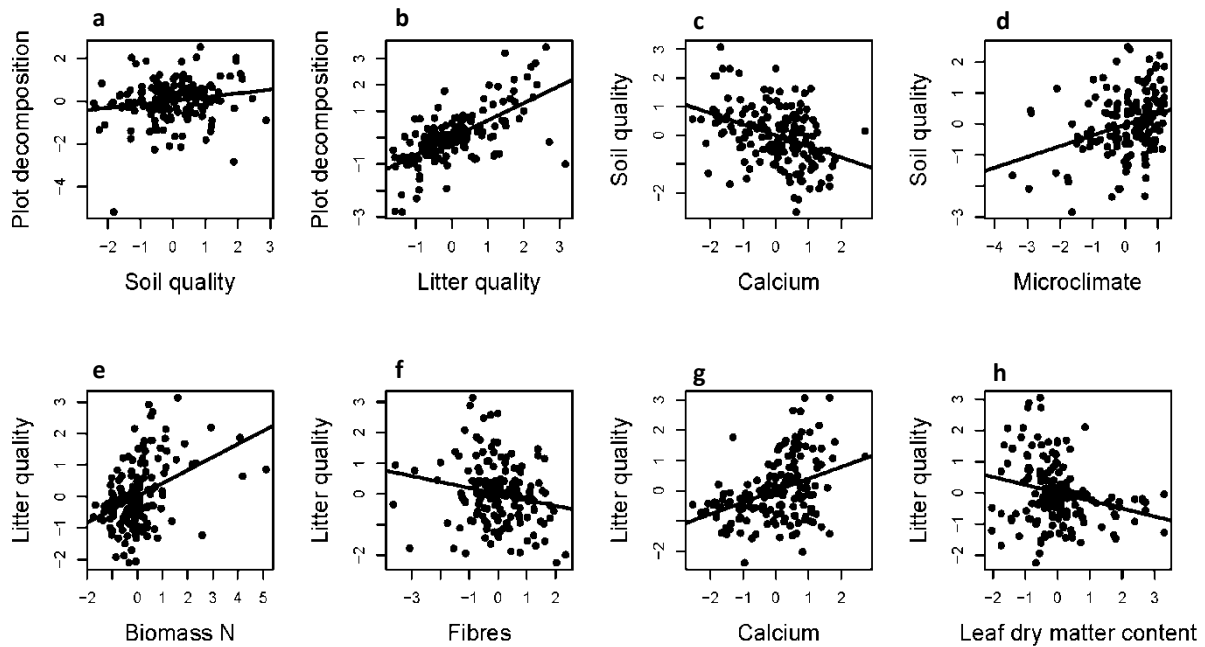
409 addition, N enrichment and plant species richness had opposite (positive and negative, respectively)  
410 indirect effects on litter quality because they had opposing effects on the N content of the biomass.

411 Plant species richness increased soil quality, as observed in the mixed models (see Fig. 1b). However,  
412 this effect was not direct or through effects on soil fauna, but was indirect and mediated by a change  
413 in microclimatic conditions: increased plant cover in diverse communities presumably increased soil  
414 moisture which increased the decomposition rate. N enrichment also increased soil quality indirectly,  
415 through a change in the microclimatic conditions.

416 Plant functional composition also altered soil quality through changes in microclimatic conditions.  
417 Communities with high Ca contents had higher plant cover and therefore higher soil quality. Ca-rich  
418 communities were dominated by herbs, which would explain this increase in cover, as herbs  
419 established better than grasses at the start of the experiment probably due to higher drought  
420 resistance. Surprisingly, however, biomass N was negatively related to plant cover. This can be  
421 explained either by a larger investment of the more productive plants in structural tissues (higher fibre  
422 contents and a dilution of biomass N content), or by the dry conditions in the first year of the  
423 experiment, which allowed the conservative species (with low N contents) to establish better than  
424 faster growing species (see Figure S8). Ca also had a direct negative effect on soil quality. Ca therefore  
425 had opposing effects on total decomposition through its effects on litter quality (positive) and effects  
426 on soil quality (negative), with a total positive effect of 0.20. Biomass N increased, and LDMC and SLA  
427 decreased, the effect of the macrofauna on decomposition, i.e. the relative differences in  
428 decomposition rate in big compared to small bags (coeff. 0.31; -0.22 and -0.14 respectively). However,  
429 the change in the effect of the soil fauna did not influence soil quality (i.e. there is no path between  
430 soil fauna and soil quality).

431 Plant functional diversity had no significant effect on decomposition, despite the increase in the soil  
432 quality effect in mixed communities plots found in the linear models (Fig. 1b). According to the SEM,  
433 this effect seems to be mediated by mass-ratio (community-weighted traits) rather than functional

434 diversity effects per se. Functional diversity increased with species richness, which can also be due to  
435 the coding of monoculture as zero diversity.



**Figure 4.** Partial plots visualizing SEM outputs from the Figure 3 of variables effects on overall decomposition (a-b), on soil quality (c-d) and on litter quality (e-h). X-axis units are standardized values, y-axis are standardized residuals of the target explanatory variable on the remaining explanatory variables.

436

437

## 438 Discussion

439

440 Here we disentangled the key drivers of litter decomposition by using data from several litter  
441 bag experiments to compare the effects of soil and litter quality on decomposition. We use a new  
442 approach to combine data from three types of litter bag in an experiment manipulating the direct  
443 (increase in soil N) and indirect (diversity and functional composition change) effects of N enrichment.  
444 Our results show that both litter and soil quality affect overall decomposition, but that litter quality is

445 most important. The key determinant of litter quality was the functional composition of the plant  
446 community, which played a bigger role than plant species richness or functional diversity. It was  
447 important to consider effects of multiple mechanisms and pathways because we found that some  
448 factors had contrasting effects on soil and litter quality (like Ca), or contrasting direct and indirect  
449 effects (species richness, biomass N), meaning that we would have missed many effects if we had  
450 looked only at their overall effects on decomposition. Therefore, N enrichment increases  
451 decomposition, mostly through indirect effects arising from a shift in functional composition towards  
452 faster growing plant species which produce easily decomposable litter.

453

#### 454 **The relative importance of litter and soil quality in determining decomposition**

455         The overall decomposition rate was more influenced by litter quality than by soil quality in our  
456 experiment (see Figure 3). This result agrees with studies in multiple biomes showing that litter traits  
457 are more important than the complexity of the decomposer community (García-Palacios, Maestre,  
458 Kattge, & Wall, 2013) or soil properties in determining decomposition (García-Palacios et al., 2016b).  
459 However, other studies in boreal forests experiencing long term N enrichment have found opposing  
460 patterns (Maaroufi et al., 2017). Part of this variation between the outcomes of these studies might  
461 be explained by differences in the relative importance of litter versus soil quality across biomes. We  
462 might expect that soil quality would be more important in unproductive ecosystems, where soil biota  
463 are expected to react more strongly to a change in microclimatic conditions (Blankinship et al., 2011).  
464 The soil quality effect could also be stronger when N enrichment leads to a decrease in soil pH, which  
465 reduces soil community diversity and abundance (Chen, Lan, Hu, & Bai, 2015; Tian & Niu, 2015). These  
466 previous studies also used different approaches to quantify litter and soil effects on decomposition  
467 and some of the variation among them may arise because they analysed different litter traits or  
468 incorporated different measures of the soil community. By combining our different litter bag  
469 experiments, we integrate all aspects of litter quality and soil quality together, allowing us to robustly

470 test for their relative importance without the need for a complete list of all the litter and soil properties  
471 that could affect decomposition. Further studies using our approach could compare the effects of soil  
472 and litter quality on decomposition across environmental gradients to determine the global  
473 importance of these factors in determining litter decomposition.

474

#### 475 Functional composition is the main driver of litter quality

476 The main determinants of litter quality in our experiment were related to the leaf economics  
477 spectrum. Plant communities with an N-rich biomass, low fibre content and low LDMC produced the  
478 most degradable material because this type of litter is easier for the soil fauna to break down. This  
479 result agrees with a large body of literature showing that litter quality relates to leaf traits indicating  
480 a fast growth strategy, like high SLA and biomass N, low LDMC, as well as low fibre content (Cornwell  
481 et al., 2008; Reich, 2014; Freschet, Aerts, & Cornelissen, 2012). Interestingly, in our experiment, we  
482 found that nutrient contents (N and Ca) were about twice as important as structural components  
483 (LDMC and fibres) in determining litter quality (combined path coefficients of 0.61 for nutrients and -  
484 0.36 for structure). Effects of N have been shown in many studies (Garnier et al., 2004; Cornwell et al.,  
485 2008) and as pointed out in Mládková, Mládek, Hejduk, Hejcman, and Pakeman (2018), Ca and Mg  
486 content (which were highly correlated in our case) also indicate a better digestibility and a higher  
487 decomposability of the litter (García-Palacios et al., 2016a). Ca and Mg are key components of  
488 invertebrate diets and can therefore increase their abundance (National Research Council, 2005),  
489 which may explain their positive effects on decomposition. However, a high Ca content did not  
490 increase the effect of macrofauna on decomposition perhaps suggesting that high Ca is also important  
491 for microbes. In addition to the nutrients, litter structural components were important in determining  
492 decomposition. We found that fibre content was important alongside LDMC in determining  
493 decomposition which suggests that there are several aspects of plant structure that matter. The fibre  
494 content, measured in bulk biomass, added complementary information on structure, as some species

495 had a low LDMC but still produced fibrous stems (see Figure S9). We did not measure plant defence  
496 compounds such as tannins and phenolics, which can also be important determinants of litter quality  
497 (Hättenschwiler & Jørgensen, 2010), however, these may correlate strongly with SLA if growth-  
498 defence trade-offs are widespread (Blumenthal et al., 2009). Overall, our results show that nutrients  
499 and structure are the key determinants of litter quality but that several different aspects are important  
500 and should be considered, as single traits may not provide adequate proxies of overall litter quality.

501 Litter diversity, calculated from the diversity of functional traits of the species present in the plot, did  
502 not have any effect on litter quality. Functional diversity might be of importance only in communities  
503 containing legumes, where a transfer of nutrients from the N-rich legume litter to more recalcitrant  
504 litter can increase decomposition (Handa et al., 2014). Our experimental design, which did not include  
505 legumes, may therefore have underestimated the effects of diversity on decomposition rates. Our  
506 results do however, agree with other studies using tree leaf litter which showed that functional  
507 composition is usually a good predictor of litter decomposition rate and that functional diversity is of  
508 secondary importance (see Finerty et al., 2016 and Bílá et al., 2014).

509

510 Soil quality and soil fauna effects are indirectly mediated by biomass Ca content and  
511 microclimate

512 Soil quality also affected the overall decomposition rate, although it was less important than  
513 litter quality. Soil quality was influenced by two factors: biomass Ca and microclimatic conditions. We  
514 observed no direct effect of N enrichment, plant species richness, functional diversity or soil fauna on  
515 soil quality, all their effects were mediated through changes in plant cover (microclimate; see Figure  
516 2). The key indicator of increased plant cover was biomass N, which suggests that a decrease in plant  
517 cover under N enrichment could decrease soil decomposition potential by decreasing humidity. N  
518 addition had both direct (positive) and indirect effects (through increasing the negative effect of  
519 biomass N) on plant cover. As microclimate had no impact on the relative effect of macro vs.



520 microfauna it seems likely that an increase in humidity was of equal importance for all soil  
521 decomposers. In contrast to the positive effects of microclimate, biomass Ca reduced soil quality. This  
522 means that plant communities producing more digestible litter, with a higher Ca (and/or Mg) content,  
523 were growing on a soil which was poor at decomposing standard litter. Since we used a fairly  
524 recalcitrant standard litter, this result could indicate that inputs of Ca-rich litter stimulated soil  
525 communities that were less effective at decomposing recalcitrant litter. Enzymes responsible for the  
526 breakdown of resistant material have been shown to be inhibited under N enrichment (Carreiro,  
527 Sinsabaugh, Repert, and Parkhurst (2000), but see Sinsabaugh (2010)). Our results may indicate that  
528 these enzymes are also inhibited by inputs of Ca-rich litter. Our use of one standard material may  
529 therefore have underestimated some effects if there are strong interactions between litter and soil  
530 quality and future studies could consider using a range of standard litters. The various direct and  
531 indirect effects of N enrichment therefore had opposing effects on soil quality: a loss of species  
532 diversity, expected under N enrichment, would reduce soil quality but this effect would be  
533 compensated for by a direct increase of plant cover under fertilisation.

534         The relative effect of macrofauna on decomposition increased with biomass N and decreased  
535 with LDMC. The macro and mesofauna contribution to decomposition was higher, relative to the  
536 effect of microfauna, when litter contained more easily degradable material. This means that high  
537 litter quality either increased the abundance of macrofauna, such as earthworms and isopods, or their  
538 efficiency in breaking down litter. Little is known about how a change in litter quality alters the effect  
539 of different soil fauna on decomposition but we can hypothesise that macrofauna are more active  
540 when feeding on higher quality litter because they actively forage for nutrients and make them  
541 available for microorganisms (see Smith & Bradford, 2003).

542         Our study used a new experimental and analytical approach to disentangle the complex  
543 drivers of litter decomposition. However, some issues need to be considered and the most important  
544 of these is probably the relatively early stage of the experiment. Overall, the lower importance of soil

545 quality compared to litter quality for decomposition indicates either that litter quality is indeed more  
546 important than soil quality, or that the effects of N enrichment, diversity and functional composition  
547 take longer to fully change soil communities (Eisenhauer et al., 2011; Boeddinghaus et al., 2019). In  
548 particular, we might expect the plant species richness effect on decomposition to become more  
549 important in longer experiments, as the soil biotic community becomes more closely linked to the  
550 aboveground community (Eisenhauer, Reich, & Scheu, 2012). The drivers of decomposition might  
551 therefore change as communities re-assemble above and belowground.

552         In our experiment we used green litter, as green litter decomposition is an important process  
553 in grasslands managed by mowing and very little senescent plant material is present in these  
554 grasslands. However, the factors determining decomposition of dead litter may differ. Due to its  
555 higher fibre to nutrient ratio, dead litter would have taken more time to decompose and the relative  
556 importance of litter quality compared to soil quality might have been lower. Although green litter  
557 accounts for a large part of the decomposed material in semi-natural grasslands, the decomposition  
558 of dead litter is also important and separate studies would need to explore its drivers. In addition, we  
559 measured litter mass loss after 2.5 months of decomposition. While some litter bags were almost  
560 empty at the end of the experiment, we have to keep in mind that the results represent a snapshot of  
561 the decomposition process, for some plots only the early stage of decomposition. It would be  
562 interesting to determine the drivers of litter decomposition at different stages of decomposition as  
563 the relative importance of soil and litter quality, and the factors determining them, might change over  
564 time (Smith & Bradford, 2003).

565

## 566 Conclusion

567

568         Decomposition was more strongly affected by litter quality rather than soil quality under N  
569 enrichment. Aboveground plant traits related to structural composition as well as nutrient

570 concentrations were major determinants of high litter quality. This suggests that several traits are  
571 needed to properly characterise litter quality and that stem structural composition should be  
572 considered alongside leaf traits. Soil quality was mainly affected by microclimatic conditions, driven  
573 by changes in plant cover. Our study suggests that, at least for the early stages of plant material  
574 decomposition, N enrichment will directly increase decomposition rates by increasing litter N content  
575 and by increasing biomass which promotes a microclimate favouring high soil faunal activity. It will  
576 indirectly affect decomposition through a shift in plant functional composition towards faster growing  
577 species, which will increase litter quality, and through a loss in plant species richness, which would  
578 mainly decrease soil quality through a reduction in plant cover. The relative importance of different  
579 drivers of decomposition under N enrichment might vary between ecosystems and further studies  
580 could use our approach to quantify the relative importance of soil and litter quality in different  
581 contexts. Nevertheless, the large effect of plant functional composition, seen in both biomass  
582 nutrients and structural components, indicates that it is among the major drivers to take into  
583 consideration when assessing overall N enrichment effects on decomposition.

584

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592

593 Authors' contribution

594 NP, SC and EA designed and set up the PaNDiv Experiment. NP and SC collected the data. NP, NH, VHK  
595 and TK processed and analysed the NIRS samples. NP analysed the data and wrote the first manuscript  
596 with the substantial input from EA, SS and SC. All authors contributed to revisions of the manuscript.

597

598 Data accessibility

599 Once this manuscript is accepted, all the relevant data will be archived in figshare  
600 (<https://figshare.com/>).

601

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