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

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Summary

1. Nitrogen (N) enrichment has direct effects on ecosystem functioning by altering soil abiotic conditions and indirect effects by reducing plant diversity and shifting plant functional composition from dominance by slow to fast growing species. Litter decomposition is a key ecosystem function and is affected by N enrichment either by a change in litter quality (the recalcitrance of the plant material) or through a change in soil quality (the abiotic and biotic components of the soil that affect decomposition). The relative importance of soil and litter quality and how the direct and effects of N alter them remains poorly known.
2. We designed a large grassland field experiment manipulating N enrichment, plant species richness and functional composition in a full factorial design. We used three complementary litter bag experiments and a novel structural equation modelling approach to quantify the relative effects of the treatments on litter and soil quality and their importance for total decomposition.
3. Our results indicate that total decomposition was mostly driven by changes in litter quality rather than soil quality. Litter quality was affected by the nutrient contents (N and calcium) and structural components of the litter (leaf dry matter content, fibres). N enrichment increased litter decomposition mostly indirectly through a shift in functional composition toward faster growing plant species producing higher quality litter. N enrichment also had effects on soil, by directly and indirectly affected vegetation cover, but this had relatively few consequences for the total decomposition rate.
4. *Synthesis*. Our approach provides a mechanistic tool to test the drivers of litter decomposition across different ecosystems. Our results show that functional composition is more important than richness or soil quality in determining litter decomposition and that N enrichment effects mainly occur via above- rather than belowground processes. This highlights the importance

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

Introduction

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

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

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

N enrichment is likely to directly and indirectly alter litter quality and therefore decomposition rates. Litter quality is largely determined by its chemical properties (nutrient contents and the presence of defence compounds) and by physical factors such as leaf dry matter and fibre contents (Garnier et al., 2004; Cornwell et al., 2008). With higher soil N availability, plants will produce more rapidly degradable tissues with higher N contents and fewer fibres. In addition to N, macronutrients like Ca and Mg may also influence litter decomposability (García-Palacios, McKie, Handa, Frainer, & Hättenschwiler, 2016a) and their availability could also be altered by N addition (Aber et al., 1998). Indirect effects of N are also likely to be important: a shift to fast growing plant communities further enhances litter quality because fast growing plants have generally higher leaf N and lower fibre contents. Fast growing plants also invest less in defences against herbivores and pathogens (Blumenthal, Mitchell, Pysek, & Jarosík, 2009) and have fewer chemicals such as tannins that reduce 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 ± 0.61°C and mean annual
147 precipitation of 1051.78 ± 168.42 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 $ha^{-1}y^{-1}$, 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^{-2}.
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

Measuring decomposition of litter bags

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

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

The plant biomass used to fill the common garden and plot bags was collected on the field before the mowing in June 2017 (with some very unproductive plots sampled again in August in order 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.

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

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

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

Common garden, Standard and Plot bags

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

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

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

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

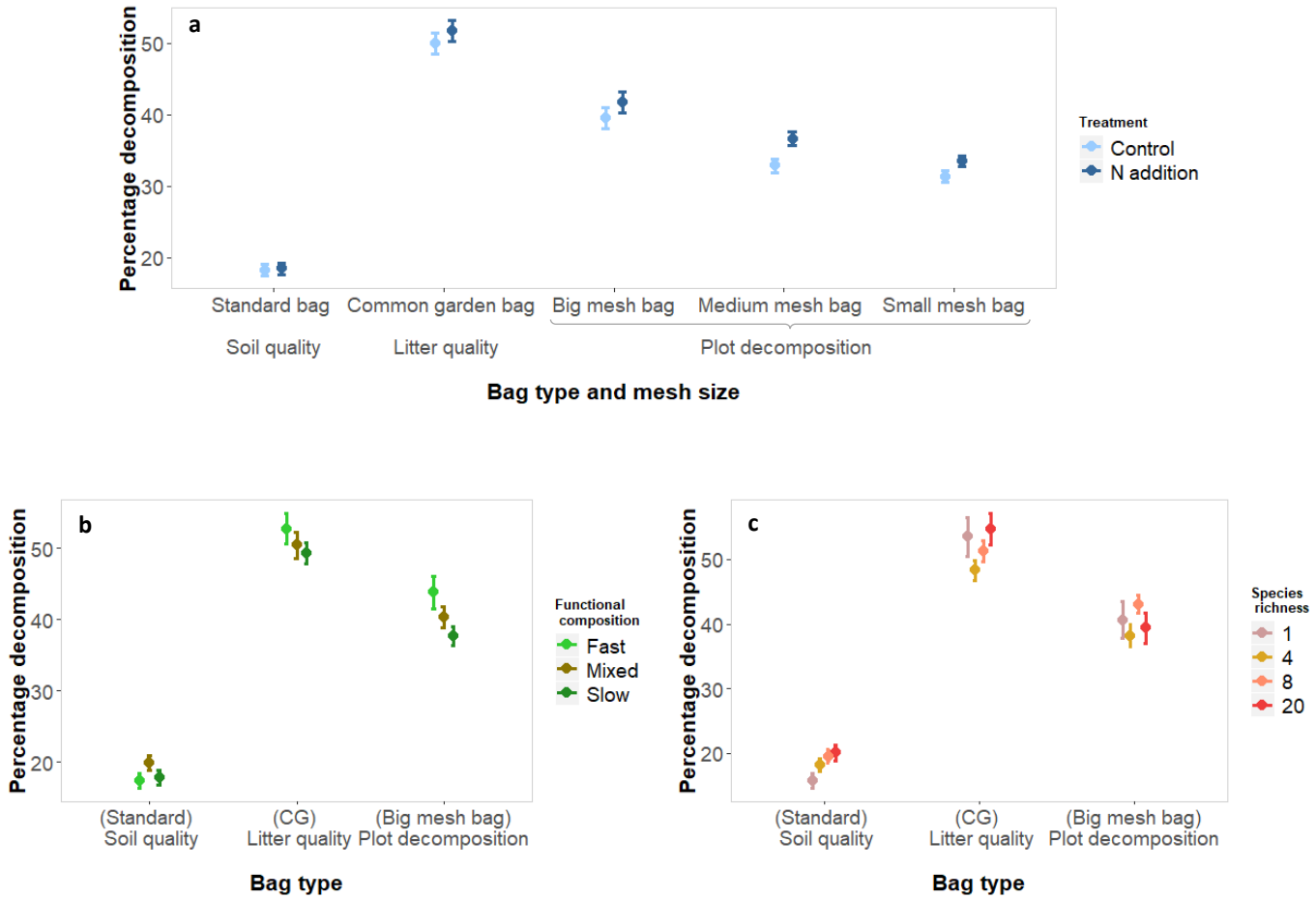

Figure 1. Effect of nitrogen addition (**a**), functional composition (**b**) and species richness (**c**) on litter decomposition depending on the litter bag type (standard, common garden and plot decomposition) and the mesh size (big, medium and small). Mean and standard error of the raw values (168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

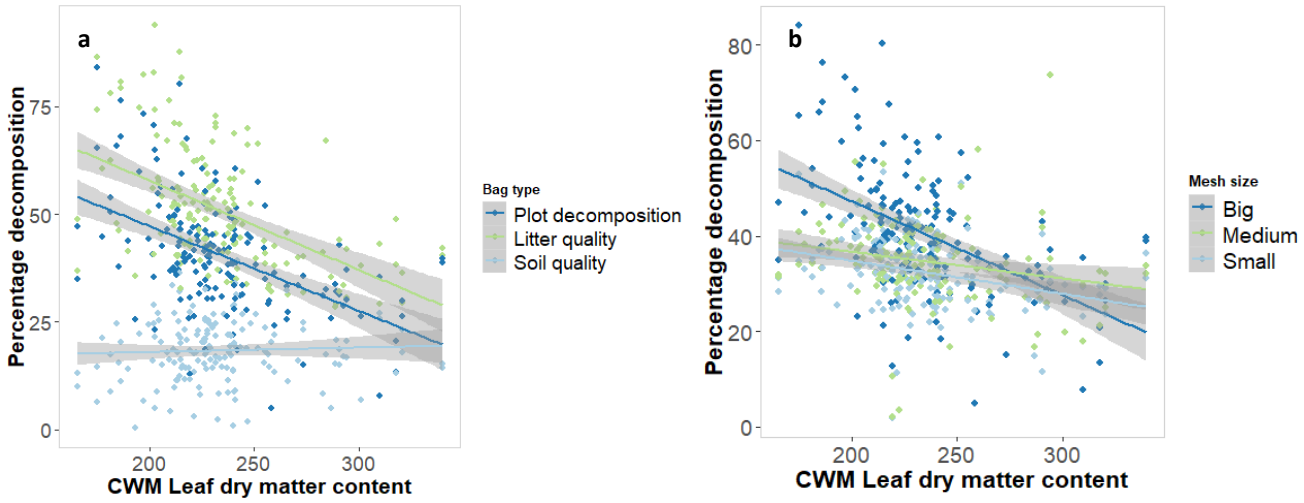

Figure 2. Effect of community weighted mean leaf dry matter content (mg g^{-1}) on decomposition depending on the bag type (**a**) and on the mesh size (**b**). Mean and standard error of the raw values (168 plots per bag).

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

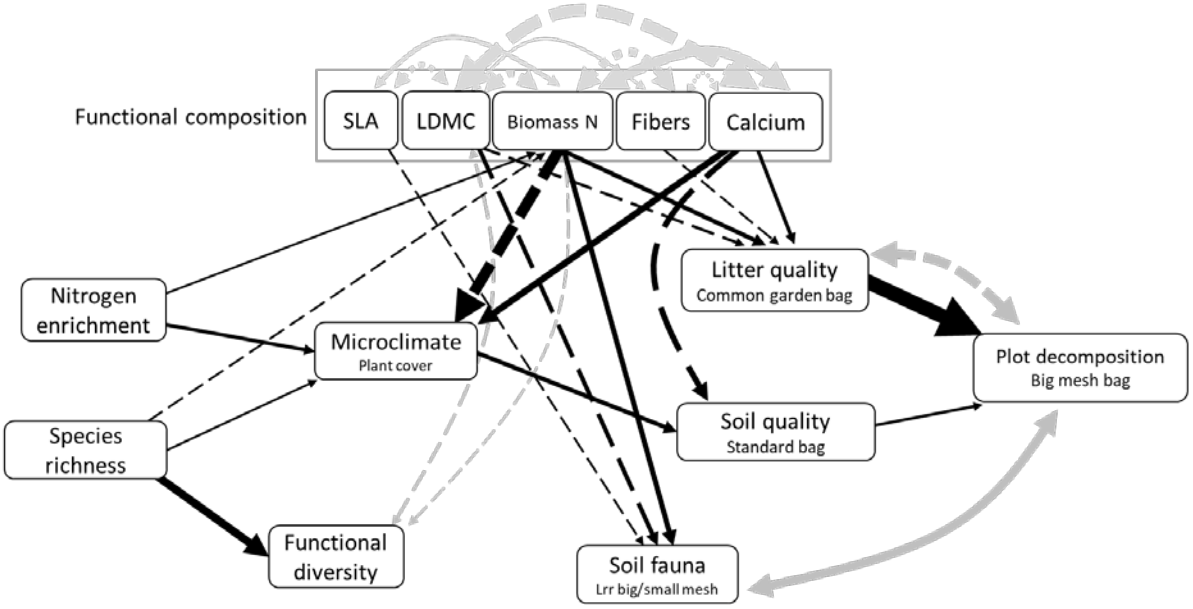

Figure 3. Final results of the structural equation model, showing effects of nitrogen enrichment, plant species richness and plant functional composition on decomposition. Dashed arrows show negative, full arrows positive path coefficients. The arrow size is proportional to the path coefficient. Double-headed 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

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

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

Plant functional composition also altered soil quality through changes in microclimatic conditions. Communities with high Ca contents had higher plant cover and therefore higher soil quality. Ca-rich communities were dominated by herbs, which would explain this increase in cover, as herbs established better than grasses at the start of the experiment probably due to higher drought resistance. Surprisingly, however, biomass N was negatively related to plant cover. This can be explained either by a larger investment of the more productive plants in structural tissues (higher fibre contents and a dilution of biomass N content), or by the dry conditions in the first year of the experiment, which allowed the conservative species (with low N contents) to establish better than faster growing species (see Figure S8). Ca also had a direct negative effect on soil quality. Ca therefore had opposing effects on total decomposition through its effects on litter quality (positive) and effects on soil quality (negative), with a total positive effect of 0.20. Biomass N increased, and LDMC and SLA decreased, the effect of the macrofauna on decomposition, i.e. the relative differences in decomposition rate in big compared to small bags (coeff. 0.31; -0.22 and -014 respectively). However, the change in the effect of the soil fauna did not influence soil quality (i.e. there is no path between soil fauna and soil quality).

Plant functional diversity had no significant effect on decomposition, despite the increase in the soil quality effect in mixed communities plots found in the linear models (Fig. 1b). According to the SEM, 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.

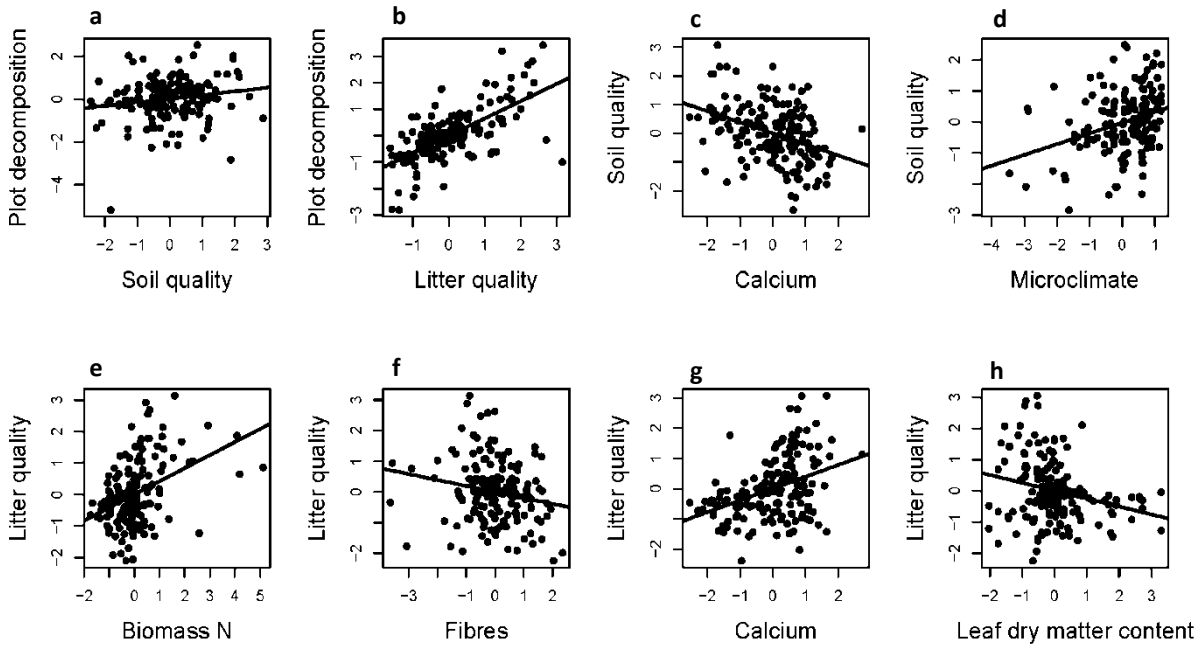

Figure 4. Partial plots visualizing SEM outputs from the Figure 3 of variables effects on overall decomposition (**a-b**), on soil quality (**c-d**) and on litter quality (**e-h**). X-axis units are standardized values, y-axis are standardized residuals of the target explanatory variable on the remaining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

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

Functional composition is the main driver of litter quality

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

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

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

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

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

Authors' contribution

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

Data accessibility

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

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