Short communication

# Litter quality impacts on grassland litter decomposition are differently dependent on soil fauna across time

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## Abstract

The main factors controlling decomposition rate are climate, litter quality and soil organisms. We investigated how decomposition was affected by interactions between litter quality and the composition of the soil community. To do this, we designed an experiment using the litterbag technique and three grass species for which a gradient of four distinct litter qualities (defined as initial nitrogen content) had been generated. We manipulated the soil community composition using different mesh sizes to constrain access of specific soil fauna to the litter on the basis of body size. Litter of a single species and quality was placed into litterbags of each of four different mesh sizes (0.1, 2, 2.8 and 4.7 mm) and bags were retrieved from the field after 30 and 60 days. Whether litter quality was a significant determinant of litter decomposition rate was dependent on both the soil community composition and length of field exposure. After 30 days there was a significant positive relationship between litter quality and decomposition for the most complex community (coarsest mesh size). The strength of this relationship declined with decreasing mesh size and, for the most restricted community (smallest mesh size), no quality–decomposition relationship was apparent. In contrast, after 60 days, decomposition was most strongly related to litter quality in the smallest mesh size bags and the relationship between quality and decomposition in the two coarsest mesh bags was non-significant. The pattern of these interactive effects between litter quality, soil community composition and time was consistent across the three grass species. We hypothesize that the effect of litter quality on mass loss within a specific mesh size was dependent on time because, while soil organisms of all size-classes responded positively to increased litter quality, they did so at a rate dependent upon their mobility.
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## 1. Introduction

The effects of plant litter quality on soil biota regulate the extent to which the soil biota in turn facilitate the decomposition of plant litter (Wardle and Lavelle, 1997). This relationship implies that interactions between litter quality and soil biota will potentially influence decomposition rate at a site. While studies that assess the role of litter quality (Melillo et al., 1982; Taylor et al., 1989; Aerts, 1997) or soil

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organisms (Curry, 1969; Setälä et al., 1996; Heneghan et al., 1999) as determinants of decomposition rate are common, studies that investigate interactions between these two variables are not. Coûteaux et al. (1991) exposed litters of differing quality to soil communities of increasing complexity and initially observed no interaction effects between quality and community complexity on decomposition. However, by the later stages of decomposition the poorer quality litter (produced under elevated $CO_2$) decomposed more slowly than the control litter with the simplest community (microflora + Protozoa) and more rapidly with the most complex community (microflora + Protozoa + nematodes + Collembola + isopods). Similarly, Bradford et al. (2002) showed that the relative effects of soil community size-class complexity on the decomposition of green and senesced litters (which differed in quality) were consistent across the early stages of decomposition. In contrast, Lukumbuzya et al. (1994) showed that litters produced under fertilisation with base-cations (Ca, K and Mg) initially decomposed faster than control litters when exposed to a complex soil community (manipulated by litterbag mesh size). These differences disappeared as decomposition of litters exposed to the complex community progressed but instead became apparent for litters exposed to the restricted community. The findings of Cornelissen (1996) for the decomposition of different tree litters further confuses the picture, with no mesh size by quality effects being apparent at either early or later stages of decomposition.

The inconsistencies between the reported interactive effects of litter quality and soil community composition on litter decomposition may be attributable to a number of factors that differed between the experiments. First, litters were placed in different environments (simple or complex microcosms, field soils, artificial soils in the field) but it is not obvious why that should alter the relative interactive effects of quality and soil community composition. Second, litter quality was manipulated in different ways ($CO_2$, multiple base-cation fertilisation, senescence, species differences) and this may have altered different quality-related variables that impact on decomposition differently. Further, even within an experiment the manipulations may have altered multiple litter quality variables (e.g. see Lukumbuzya et al., 1994). Given the complex nature of the decomposi-

tion process, with a range of different quality related variables influencing decomposition rate positively or negatively over time (Swift et al., 1979), classification of litter as being of "higher" quality is thus somewhat arbitrary. We manipulated litter quality of three grass species in a highly controlled manner in an attempt to produce litters that differed only by initial nitrogen concentration (or C:N ratio), a quality variable that is generally a good predictor of the rate of early decomposition (Taylor et al., 1989). Litters of a single quality and species were placed in litterbags of four different mesh sizes and then mass loss from these bags was followed in the field across 60 days. We hypothesised that higher quality litters would decompose more rapidly, and that this effect would occur earlier when larger fauna were present because they are more mobile than smaller organisms and so potentially can colonise litter more rapidly.

## 2. Materials and methods

### 2.1. Study site

The experiment was conducted at Silwood Park, UK (51°22′N, 00°37′W) in a semi-natural acid grassland (soil pH 5.26 in 0.01 M $CaCl_2$) fenced since 1998 to protect against rabbit and deer. The site had an altitude of 50 m a.s.l. and a mean annual rainfall of 652 mm. The soil was a sandy loam with a clay:silt:sand ratio of 10:8:82 wt.%. Experimental plots were free of herbicide, pesticide and fertiliser application (see Hector et al., 2000 for a full description).

### 2.2. Litter preparation

Monocultures of *Arrhenatherum elatius* L., *Holcus lanatus* L. and *Agrostis capillaris* L. were grown under controlled light, temperature and humidity in 25 cm diameter pots filled with nutrient poor, sandy soil. The three species were chosen as they are common in UK grasslands and were found in the experimental site. Twice a week, for 10 weeks, each pot received 18.75 µl of modified Hoagland's solution in 1 l of water, ensuring complete control of nutrient supply to the plants (Cotrufo et al., 1995). To manipulate plant nitrogen content, $NH_4NO_3$ in the solution was added at four different concentrations: 5.5, 55,

Table 1
Nitrogen concentration (N) and carbon:nitrogen ratio (C:N) of the aboveground tissue of the three grass species used in litterbags

| Species | Quality | N (wt.%) | C:N |
|---------|---------|----------|-----|
| *Arrhenatherum elatius* | A | 1.83 | 23:1 |
| | B | 2.24 | 20:1 |
| | C | 2.71 | 16:1 |
| | D | 3.45 | 13:1 |
| *Holcus lanatus* | A | 1.84 | 23:1 |
| | B | 1.95 | 22:1 |
| | C | 2.28 | 19:1 |
| | D | 2.80 | 15:1 |
| *Agrostis capillaris* | A | 1.91 | 22:1 |
| | B | 2.45 | 18:1 |
| | C | 3.04 | 15:1 |
| | D | 3.50 | 13:1 |

150 and 330 mg N l$^{-1}$. This produced four distinct plant tissue qualities which, hereafter, will be referred to from lowest to highest quality as A, B, C and D, respectively (Table 1).

To ensure a strongly pronounced quality gradient, aboveground tissue was harvested green and then dried at 40 °C. Total C and N content of the litter was determined by coupled combustion chromatography analysis (NRM Ltd., Bracknell, UK).

### 2.3. Litterbag construction

Litterbags (9 cm × 8 cm) were constructed from nylon mesh (Northern Mesh, Oldham, UK) with four different pore sizes. The mesh sizes were designed to permit entry of microfauna only (100 μm mesh), microfauna and mesofauna (2 mm mesh), microfauna, mesofauna and small macrofauna (2.8 mm mesh), and microfauna, mesofauna and all macrofauna (4.7 mm mesh). Bacteria and fungi had access to all litters. Individual bags were filled with 0.9–1.1 g of litter; bag edges were heat-sealed.

### 2.4. Experimental design

Two litterbags per species of each quality and mesh size were incorporated into the litter layer in each of five 3 m × 3 m blocks on 22 May 2001. One bag was retrieved after 30 and 60 days. In total, 480 litterbags were used. Dry (40 °C) mass of remaining litter was determined after separation from foreign material (soil, shoots, roots, fauna). We refer to mass loss from the bags as "decomposition" but recognise that in the strictest sense, this mass loss is the result of both the physical process of "breakdown" and the catabolic process of "decomposition" (Anderson, 1973; Bradford et al., 2002).

### 2.5. Statistical analysis

Analysis of variance (ANOVA) was used to investigate how litter quality interacted with soil community composition to determine litter decomposition rate over time. A full interaction linear model with three fixed, discrete factors (mesh size, species and removal time) and one fixed, continuous factor (quality defined as initial nitrogen concentration) was constructed. Block was included as a non-interacting factor. Data were expressed as "proportion of litter mass remaining" and were arcsine square-root transformed to meet the assumptions of ANOVA.

## 3. Results

Litter quality, soil community and removal time had a significant three-way interaction effect on litter mass loss ($P < 0.001$; Table 1), indicating that the effect of litter quality on the pattern of mass loss over time differed for different soil community compositions. Although the higher order interaction with species was not significant ($P > 0.05$; Table 2), indicating that the combined effects of quality, soil community and removal time on mass loss were similar across species, we did not pool species data for later analyses. This was because there was also a significant three-way interaction between soil community, species and removal time ($P < 0.05$; Table 2), suggesting that mass loss from the different species did not behave in a consistent manner in different mesh size bags across time. Thus, to investigate the three-way interactions we quantified (using linear regression) the relationship between quality and mass loss for each mesh size and species individually (Fig. 1). This approach permitted us to address our main objective, that being to determine if litter quality effects on decomposition are consistent when litters are exposed to different soil community compositions.

Table 2
Results of the analysis of variance to investigate the interactive effects of litter quality, soil community composition, time and species identity on litter decomposition

| Source of variation | d.f. | s.s. | s.s. (%) | m.s. | $F$ | $P$ |
|---|---|---|---|---|---|---|
| Removal time ($T$) | 1 | 10258.9 | 31.5 | 10258.9 | **1430.2** | **<0.001** |
| Mesh size ($M$) | 3 | 12869.4 | 39.5 | 4289.8 | **598.1** | **<0.001** |
| Species identity ($S$) | 2 | 3005.1 | 9.2 | 1502.6 | **209.5** | **<0.001** |
| Quality ($Q$) | 1 | 1158.9 | 3.6 | 1158.9 | **161.6** | **<0.001** |
| Block | 4 | 58.5 | 0.2 | 14.6 | 2.0 | 0.088 |
| $T \times M$ | 3 | 761.2 | 2.3 | 253.7 | **35.4** | **<0.001** |
| $T \times S$ | 2 | 107.5 | 0.3 | 53.7 | **7.5** | **<0.001** |
| $M \times S$ | 6 | 430.8 | 1.3 | 71.8 | **10.0** | **<0.001** |
| $T \times Q$ | 1 | 4.1 | <0.1 | 4.1 | 0.6 | 0.450 |
| $M \times Q$ | 3 | 72.2 | 0.2 | 24.1 | **3.4** | **<0.05** |
| $S \times Q$ | 2 | 89.3 | 0.3 | 44.6 | **6.2** | **<0.01** |
| $T \times M \times S$ | 6 | 278.9 | 0.9 | 46.5 | **6.4** | **<0.001** |
| $T \times M \times Q$ | 3 | 308.3 | 0.9 | 102.8 | **14.3** | **<0.001** |
| $T \times S \times Q$ | 2 | 21.0 | <0.1 | 10.5 | 1.5 | 0.233 |
| $M \times S \times Q$ | 6 | 34.5 | 0.1 | 5.8 | 0.8 | 0.569 |
| $T \times M \times S \times Q$ | 6 | 47.4 | 0.1 | 7.9 | 1.1 | 0.361 |
| Residual | 425 | 3048.4 | 9.4 | 7.2 | | |
| Total | 476 | 32554.4 | 100 | 68.4 | | |

Significant effects ($P < 0.05$) are shown in bold.

After 30 days of exposure, mass loss generally responded more positively to increasing litter quality as mesh size was increased; no significant quality effect ($P > 0.05$) was evident for the smallest mesh size (Fig. 1). However, after 60 days of exposure the pattern was markedly different: the strongest response of mass loss to the quality gradient was observed for the smallest mesh size and no significant quality effects were observed for the two coarsest mesh sizes (Fig. 1).

## 4. Discussion

Litter decomposition rate increased with increasing mesh size (Fig. 1), supporting earlier studies that have investigated the effects of exclusion of specific soil faunal size-classes on the rate of litter decomposition (Setälä et al., 1996; González and Seastedt, 2001; Bradford et al., 2002). In addition, and in support of previous studies (Taylor et al., 1989), initial nitrogen concentration of the litter was positively related to decomposition. However, whether this relationship was significant was dependent on the litterbag mesh size and timing of litter removal (Fig. 1).

After 30 days of field exposure, and as soil macro- and then mesofauna were progressively excluded, the relationship between decomposition rate and litter quality became less pronounced. In contrast, by 60 days, decomposition was most strongly determined by quality in the bags with the least complex community and, when the most complex community had access to the litter there was a null quality–decomposition relationship (Fig. 1). We suggest that at the 30 day removal point that larger soil fauna generated the marked quality–decomposition relationship because they actively seek and feed on high quality litter (Satchell and Lowe, 1967; Kohli et al., 1999). Further, larger fauna may make the litter more accessible to bacteria and fungi (Petersen and Luxton, 1982), promoting microbial growth and therefore decomposition further (Lukumbuzya et al., 1994). We postulate that a null quality–decomposition relationship occurred after 60 days in the coarser mesh bags because initial nitrogen concentration of the remaining litter (only 20–30% of the original) was no longer controlling decomposition. It is entirely feasible that a variable such as lignin content was controlling decomposition at this time (Melillo et al., 1982; Taylor et al., 1989); we did not deliberately vary the lignin content of our
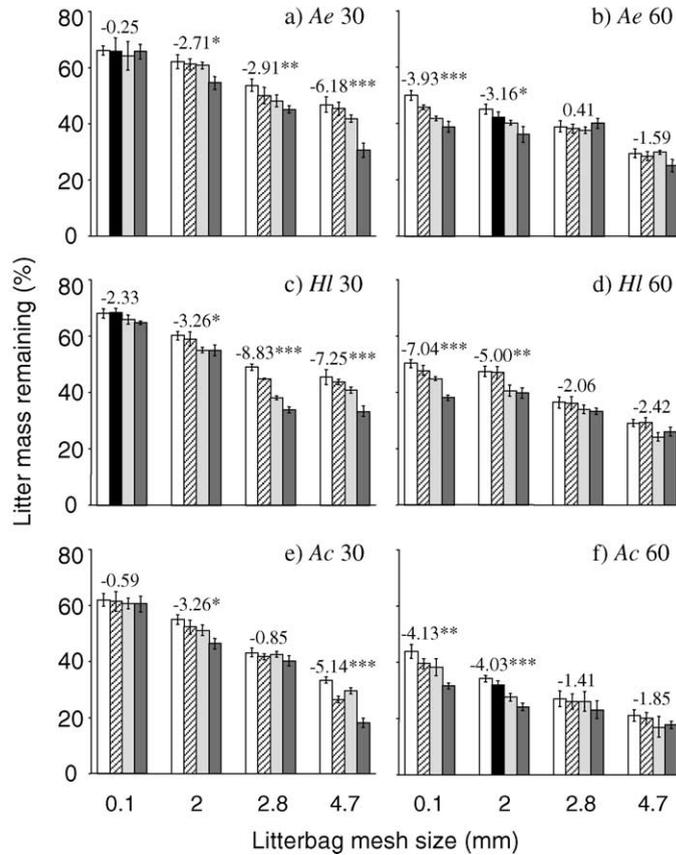
Fig. 1. Effect of litter quality on mass loss of (a, b) *Arrhenatherum elatius*, (c, d) *Holcus lanatus*, (e, f) *Agrostis capillaris* litter after 30 days (a, c, e) and 60 days (b, d, f) of field exposure to a gradient of soil community size-class complexity. Mass remaining (mean ± 1 S.E.; percentage of original) in litterbags of four different mesh sizes is shown. Litter quality was manipulated to generate a gradient from low (A; white bars) to high quality (D; dark grey bars). Means and standard errors were calculated from arcsine square-root transformed data at the plot level ($n = 5$) and then back transformed. The linear relationship between mass remaining (transformed data) and litter quality within a mesh size is shown by the slope coefficient and significance level (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

litters and so would not have expected an interactive effect between quality and the soil community for later stages of decomposition. However, when interpreting our results it must be remembered that we only assessed the total C and N contents of our litters—other quality-related variables may have been unintentionally varied, although our findings do not suggest that this was the case.

Why did a significant quality–decomposition relationship only become apparent in the smallest mesh bags after 60 days? We suggest that the microbiota were responding to the quality gradient at 30 days but that the response took 60 days to become apparent because the microbiota rely more on growth than mobility to colonise litter, producing a lag in the observed response. Further, we suggest that this lag-time was exacerbated because litter-colonisation was not facilitated by the action of litter-shredders. We consider that mesofauna fall within the growth–movement response continuum between microbiota and macrofauna, which is why a significant quality–decomposition relationship was observed for the 2 mm bags at both removal points. Given that mesofauna are the dominant litter-shredders (Petersen and Luxton, 1982) and that in the absence of macrofauna litter may decompose more slowly (Bradford et al., 2002), there may

also have been more time for microbiota to exert an effect on the relationship in the mesofauna bags.

We assessed the relationship between litter quality and decomposition rate for each time-point and mesh size individually (Fig. 1). For this reason, many of the caveats associated with interpretation of litter loss from different mesh size bags (e.g. different microenvironments within different mesh size bags; Vossbrinck et al., 1979) do not impinge upon our interpretation of the interactive effects between quality and soil community composition. However, it is feasible that leaching could provide a mechanism whereby a significant quality–decomposition relationship would appear earlier in larger mesh size bags and then later in smaller mesh bags (Anderson, 1973). We used regression analysis to test whether the total initial nitrogen concentration of litter was a predictor of decomposition in a related study which used the same litters, site and timing as the current study but where litters were instead mixed within 2.8 mm mesh bags (Smith and Bradford, 2003). There was no significant relationship between nitrogen concentration and decomposition (data not shown), supporting the contention that soil biota, and not leaching, were responsible for the litter quality by mesh size interactions we observed.

It is important to note that our study was a fundamental investigation of potential interactions between a litter quality variable recognised as a good general predictor of the initial stages of decomposition (Taylor et al., 1989) and soil community composition. We used green litter to enable a marked gradient in litter nitrogen concentration and this may decompose in a quantitatively and qualitatively different way to senesced litter (but see Bradford et al., 2002). Also, we manipulated soil community composition by constraining access to litter by organism body size: such a manipulation does not necessarily simulate how soil community composition will respond temporally, spatially or in response to disturbance. Future work must assess how soil community composition, manipulated in a more realistic manner, affects decomposition of senesced litters (where quality-related variables are controlled to some extent) in both the short- and long-term. In conclusion, we observed strong interactive effects of litter quality and soil community composition on decomposition and the specific manifestation of these effects was dependent on the time of litter exposure.

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