Temporal dynamics of biotic and abiotic drivers of litter decomposition

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Abstract

Climate, litter quality and decomposers drive litter decomposition. However, little is known about whether their relative contribution changes at different decomposition stages. To fill this gap, we evaluated the relative importance of leaf litter polyphenols, decomposer communities and soil moisture for litter C and N loss at different stages throughout the decomposition process. Whereas both microbial and nematode communities regulated litter C and N loss in the early decomposition stages, soil moisture and legacy effects of initial differences in litter quality played a major role in the late stages of the process. Our results provide strong evidence for substantial shifts in how biotic and abiotic factors control litter C and N dynamics during decomposition. Taking into account such temporal dynamics will increase the predictive power of decomposition models that are currently limited by a single pool approach applying control variables uniformly to the entire decay process.

Introduction

Climate and litter quality (chemical and physical composition) are the predominant drivers of litter decomposition at large spatial scales (Parton *et al.* 2007; Cornwell *et al.* 2008; but see Bradford *et al.* 2015). Decomposer communities (microbes and fauna) can explain part of the residual variance in global litter decomposition (Wall *et al.* 2008; García-Palacios *et al.* 2013), but they can also play a major role at smaller spatial scales (Coq *et al.* 2010; Bray *et al.* 2012). The majority of previous studies evaluated decomposition as a single-pool exponential model estimating a uniform decomposition rate constant (*k*) based on several sequential harvests of decomposing litter. By taking this approach, the dynamic process of litter decomposition is expressed as a univariate metric, strongly facilitating the assessment of how *k* might be influenced by a range of different factors. However, at the same time it considerably limits the evaluation of temporal dynamics (Adair et al. 2010) and the assessment of how the relative importance of biotic and abiotic drivers may shift during the course of decomposition.

Three existing main gaps still limit a rigorous assessment of the temporal dynamics in decomposition. First, compared to the widely measured decomposition rates, we know surprisingly little about how litter quality changes over time in decaying litter (Wickings *et al.* 2012; Parsons *et al.* 2014). It is generally assumed that initially widely different chemistry of leaf litter from distinct plant species converges during decomposition (Melillo *et al.* 1989; Preston *et al.* 2009), as a result of the increasing loss of labile compounds (e.g. carbohydrates and amino acids) and the increasing dominance of lignin. However, important differences in litter chemistry among plant species can still arise at late decomposition stages (e.g. >75 % mass loss) in the presence of contrasted communities of soil decomposers (Wickings *et al.* 2012). Second, microbes, the ultimate actors in the litter decay process, and invertebrates (e.g. nematodes), undergo major

successional changes during the decomposition process (Wang *et al.* 2004; Vořišková & Baldrian 2013). Due to practical and technical reasons, most studies assessing the role of decomposers on litter decay have used litterbags of different mesh sizes to exclude particular taxa based on body size. Such a black-box approach usually excludes a detailed analysis of the large biodiversity found in soils, and the assessment of community shifts through time (van der Wal *et al.* 2013). Finally, the third area of limited knowledge concerns the role of climate that is usually evaluated using long-term averages from weather stations or interpolations from global databases (Parton et al. 2007; Wall *et al.* 2008). While this approach may be acceptable over very large spatial scales, it is clearly oversimplifying the strong impact of local scale variation in climatic conditions, which may lead to erroneous conclusions about climate control over decomposition (Bradford *et al.* 2014, 2015). Moreover, the relative importance of local scale climate in controlling decomposition is likely to differ during contrasting stages of the litter decomposition process.

Plant leaf litter can contain considerable amounts of polyphenols such as monomeric phenolic compounds (e.g. phenolic acids and flavonoids) or polymers (e.g. condensed tannins) (Horner *et al.* 1988). The labile proportion of polyphenols is usually highly soluble in water and thus rapidly lost from litter through leaching. On the other hand, tannins can form stable recalcitrant complexes with proteins (Horner *et al.* 1988) that are difficult to access by decomposers (Wurzburger *et al.* 2009). Despite these changes in the proportion of monomeric phenolic compounds *vs.* tannins over time, its consequences for litter decomposition are basically unknown. For instance, polyphenols are usually measured only in the initial litter, and decomposition has shown contrasted relationships, positive with phenolics (Hättenschwiler & Bracht Jørgensen 2010) but negative with condensed tannins (Coq *et al.* 2010). Particularly, the formation of tannin-

protein-complexes can inhibit microbial processes such as decomposition by affecting microbial activity (Schimel *et al.* 1998), or by changing microbial community composition (Baptist *et al.* 2008). Polyphenols can also influence litter decomposition through effects on soil fauna. Nematodes, the most abundant group of soil animals (Coleman & Crossley 1996), are usually negatively affected by high concentrations of polyphenols in plant tissues. While this role has been extensively explored for plant resistance to pathogens (Bennett & Wallsgrove 1994; Ohri & Pannu 2010), the implications for litter decomposition are unknown. Although nematodes do not directly feed on litter, the occurrence of different nematode functional groups (e.g. fungal and bacterial feeders) can have an important effect in litter decomposition via microbial grazing (Coleman & Crossley 1996).

Our main goal was to assess if and how the relative importance of abiotic and biotic drivers of litter carbon (C) and nitrogen (N) losses change at different stages of the decomposition process. To do so, we measured litter C and N losses, litter decomposer communities (microbes and nematodes), litter polyphenols (total phenolics and condensed tannins), and local-scale climatic conditions (soil temperature and moisture) successively during the decomposition of high and low litter quality mixtures exposed in five distinct forest sites in southern France. We hypothesized that i) litter C and N loss monotonically increase over time, and higher litter quality mixtures show higher losses of litter C and N than low litter quality mixtures (Cornwell *et al.* 2008), ii) litter polyphenol concentrations rapidly decrease over time, promoting litter chemical convergence (Parsons *et al.* 2014), and iii) litter decomposers track converging litter chemistry temporal patterns, resulting in more similar communities over time (Baptist *et al.* 2008). Following these three hypotheses, we also hypothesized that iv) biotic control over C and N losses predominates in initial stages of decomposition, but that abiotic

control gains in importance during later stages of decomposition as a result of converging litter chemistry and decomposer communities.

Materials and methods

Study sites, experimental design and litterbag field incubation

The experiment was conducted at five forest sites in southern France covering a large regional gradient in altitude and climatic conditions (Table 1). All sites had similar slopes and aspects, and a closed tree canopy dominated by *Fagus sylvatica* L. Freshly fallen leaf litter from three woody species (*Fraxinus angustifolia* Vahl., *Pistacia terebinthus* L. and *Alnus glutinosa* L.) was collected in autumn 2012 in forests 30 km north-west of Montpellier, France. We selected these three species, because they represent a wide range in litter quality (De Oliveira *et al.* 2010; Handa *et al.* 2014), and because none of the three species were present at any of the five study sites, avoiding potential home-field advantage effects, and thus facilitating cross-site comparisons.

We constructed 20 × 20 cm litterbags filled with 10 g of 40 ºC-dried leaf litter. We included a high litter quality mixture (*A. glutinosa* + *F. angustifolia*) with lower C:N and lignin:N ratios, and lower concentrations of polyphenols, than the low litter quality mixture (*A. glutinosa* + *P. terebinthus*) (Table S1). Litter mixtures were included to represent realistic litter layers, as litter in undisturbed forest floors typically consists of multiple species, which in turn can drive interactions among microbes and invertebrates (Gessner *et al.* 2010). All litterbags (0.6 × 0.5 mm bottom side, 8 × 5 mm top side) were placed on the forest floor in July 2013. We selected four homogeneous areas (blocks) at each site. One replicate of the two litter qualities (high and low) were distributed in each of the four blocks according to a randomized block design. We placed a total of three

litterbags per litter quality level in each block for three successive harvests at 3, 7 and 11 months of field incubation, resulting in a total of 120 litterbags.

Local-scale environmental conditions

Surface soil (5 cm depth) temperature and moisture were continuously monitored at each site using automated sensors (RT-1 and EC-5 soil temperature and moisture sensors, respectively, Decagon Devices Inc., Pullman, USA, Fig. S1). For determining soil characteristics, we randomly took three soil cores (5 cm diameter, 10 cm depth) within each block during litterbag installation. Soil cores were bulked by block, sieved at 2 mm mesh, and air-dried for one month. Soil subsamples were sent to the INRA laboratory at Arras, France, for standard soil physicochemical analyses (texture, pH, total C, total N, Olsen P, NH_4^+ - N and NO_3^- - N; Table 1).

Leaf litter microbial and nematode communities

Upon litterbag retrieval, two litter sub-samples were taken from each litterbag. The functional composition of heterotrophic microbial communities was analyzed in one of the litter sub-samples (approximately 200 mg of fresh litter) with the MicroResp™ system (Macaulay Scientific Consulting, Aberdeen, UK). This method assesses the community-level physiological profiles (CLPP) by testing ecologically meaningful C sources of different chemical recalcitrance (García-Palacios *et al.* 2011). We calculated substrate induced respiration rates expressed in µg $C\text{-}CO_2$ respired g^{-1} litter h^{-1} by using the control (deionized water but no C source added) as the basal respiration. Nematodes were extracted from the second sub-sample (approximately 4 g of fresh litter) using the Baermann funnel technique (Baermann 1917). An aliquot of 20 ml of deionized water plus nematodes from each sample was collected in the same vial after 24 h, 48 h and 72

h, for a total volume of 60 ml. After extraction, the nematodes were preserved under 5 % formalin, and determined to functional group level (bacterial feeders, fungal feeders, plant parasites, omnivorous and predators) according to Yeates *et al.* (1993) using an inverted CKX41 Olympus microscope. Nematode abundance was expressed per unit of litter dry weight after correcting for litter moisture.

Litter C loss, N loss and polyphenol concentration

After removal of the subsamples used for microbial and nematode measurements, the remaining litter material was gently rinsed with tap water to remove soil particles and animal feces, dried at 60 ºC to constant mass, and weighed. The dried leaf litter material was ground to fine powder with a ball mill. Litter ash content was determined from each individual litterbag, and all litter mass loss data are expressed as ash-free litter mass. Total phenolics were measured with the Folin-Ciocalteau reagent following Marigo (1973), but using methanol (50%) as solvent instead of water. Condensed tannins were determined according to the acid butanol method (Porter *et al.* 1986). C and N concentrations were determined using a CN elemental analyser (ThermoFinnigan, Milan, Italy). Using the initial- and post-field-incubation litter mass, and the respective litter C and N concentrations, litter C and N loss (%) were calculated following Handa et al. (2014).

Statistical analyses

First, we evaluated the effects of litter quality and site on litter C and N loss, and on the litter concentrations of total phenolics and condensed tannins over time (3, 7 and 11 months) using a factorial ANOVA. Site, litter quality and incubation time were introduced in the model as fixed-effect factors, while block (site) was introduced as a random-effect factor. The effects of treatments on the microbial CLPP and nematode

functional group composition were evaluated using semiparametric permutational ANOVA-type tests (PERMANOVA, Anderson 2001). We also performed nonmetric multidimensional scaling (NMDS) for a more specific interpretation of the multivariate analyses of litter decomposer communities. To interpret significant interactions, we used a simple main effects test. Data were divided into subsets based on one of the factors of the interaction and were then subjected to ANOVA or PERMANOVA as appropriate. The Tukey's HSD test was used for post-hoc comparisons of factors with more than two levels.

The analyses described above assess well the differences in decomposer communities and litter chemistry over time. However, the underlying drivers of litter C and N dynamics need to be examined at consistent decay stages along the litter decomposition continuum (Wickings *et al.* 2012; Parsons *et al.* 2014). To investigate whether the importance of abiotic and biotic factors differed along consecutive decomposition stages, we used multi-group comparisons of structural equation modeling (SEM). Following current concepts of the decomposition process (see Appendix S1 for further explanations), we proposed an *a priori* model of hypothesized relationships within a path diagram (Fig. 1), allowing a causal interpretation of the model outputs (Grace 2006). We first followed a smoothing approach to allow for the determination of consecutive stages along the litter decomposition continuum. Smoothing was achieved by rounding mass loss values for each litterbag to the next 10 % (i.e. creating discrete groups of 10 % mass loss intervals). 40 % mass loss smoothing was selected for six groups (0-40, 10-50, 20-60, 30-70, 40-80 and 50-90 % mass loss). These intervals allowed including enough samples to run multi-group comparisons (a lower smoothing level included fewer samples in some of the groups). This approach, similar to time-lag analysis, is a powerful way of measuring temporal dynamics in multivariate data when

the time frames are too short to show patterns (Collins *et al.* 2000), and has been used before to address litter chemical patterns in decomposing litter (Parsons *et al.* 2014). We run a separate model for litter C and N loss.

'Litter quality' was represented as a binary variable coding for low and high quality litter mixtures. A series of independent ordinations were conducted to reduce the dimensionality of the multivariate climatic, polyphenols, and decomposers datasets. The first axis of the climatic principal component analysis (PCA) accounted for 55 % of the variance, and was significantly correlated with mean soil moisture (r = 0.84). Thus, soil temperature was not included in the SEM as it was less important than soil moisture describing the climatic variability between sites and litter incubation times. 'Polyphenols' represented the most explicative axis (91 % of the variance) of another PCA, which was significantly correlated with total phenolics (r = 0.82) and condensed tannins (r = 0.95). The litter microbial CLPP ('Microbes') and nematodes functional group composition ('Nematodes') were represented as the first axis of the two NMDS conducted to interpret the PERMANOVA results. See Appendix S1 for more information on these analyses.

Results

Changes in litter C and N loss over time

Litter C and N loss increased over time, and differed among the five sites (Fig. 2). Overall, the highest amount of C and N was lost at Sauclieres and the lowest at Lagarde d'Apt. Litter C loss through time was similar among sites, but N loss dynamics differed among sites ($P_{site \times time} < 0.001$). Separate ANOVAs conducted at each site revealed that while N loss monotonically increased over time at Sainte Baume, it did not increase any further beyond 7 months at all other sites (Fig. 2B). Separate ANOVAs at each site, conducted to interpret the significant site × litter quality interaction (Table S2), revealed higher C

and N loss in the high compared to the low litter quality mixtures at all sites, but Sauclieres ($P > 0.500$).

Changes in litter polyphenol concentrations over time

The concentrations of total phenolics and condensed tannins decreased strongly over time (Fig. 3). Separate ANOVAs at each time interval, conducted to interpret the significant litter quality × time interaction ($P < 0.001$, Table S3), showed that the loss rate of both, total phenolics and condensed tannins, was higher in the low compared to the high quality mixture, leading to similar low concentrations after 11 months despite the large differences in initial litter material. The decrease over time was consistent across sites for total phenolics (Fig. 3A), but it differed among sites for condensed tannins (Fig. 3B).

Changes in litter decomposer communities over time

A significant litter quality × time interaction was found when analyzing the microbial CLPP and the functional group composition of nematode communities ($P < 0.05$, Table S4). Separate ANOVAs conducted for each time interval revealed contrasting patterns between the two groups of decomposers. The capability of microbes to degrade most of the C substrates was larger in the high quality compared to the low quality litter mixtures (Fig. 4A). However, these differences between litter types converged after 7 and 11 months, with overall lower rates of substrate use across C substrates at the end of the litter field incubation (Fig. S2). The nematode community was similar between litter types after 3 months, but shifted towards an increased abundance of both fungal and bacterial feeders in the high litter quality after 7 and 11 months (Fig. 4B). There were also considerable differences in nematode community composition among sites after 3 months of field litter incubation, which converged later ($P_{site \times time} < 0.001$, Fig. 4B).

Interactions between climate, polyphenols and decomposers along different litter decomposition stages

Litter quality had a positive influence on N loss (i.e. higher N loss from higher quality litter) across decomposition stages (Fig. 5), but C loss was only stimulated in high compared to low quality litter in the later stages (Table S5). At late decomposition stages (40-80 and 50-90 mass loss intervals), litter quality effects were mostly a direct effect. However, in the early decomposition stages, 44 and 25 % of the total litter quality effects on C and N loss, respectively, were mediated by the joint influence of polyphenols and decomposers. Polyphenols were negatively associated with C loss with ongoing decomposition, but they had no impact on N loss at any of the decomposition stages considered. Across all litter mass loss intervals, higher concentrations of polyphenols were related to a higher capability of microbes to degrade the range of C substrates used in the CLPP assay. On the other hand, polyphenols reduced the abundance of bacterial and fungal feeding nematodes during the three first decomposition stages (0-40, 10-50 and 20-60 % mass loss; Table S5). Microbial CLPP were consistently and negatively related to litter C and N loss across all mass loss intervals, indicating lower decomposition with higher rates of C substrate use. The positive influence of soil moisture on litter C and N loss, observed at the lower and higher end of the mass loss range, indicated higher losses of both C and N with increasing mean soil moisture. Such effects were mostly direct, as indirect effects mediated by polyphenols and decomposers only represented 5% of the total soil moisture effects.

Discussion

Litter decomposition is jointly influenced by environmental conditions and community-level plant litter-decomposer interactions that vary across time and space. Consequently,

pinpointing the specific drivers of decomposition is challenging, but of major importance to accurately predict how litter decay will respond to climate change. According to the main goal of this study, we identified how the relative importance of different biotic and abiotic factors changes along different decomposition stages, ranging from initial to advanced decay of up to 90 % of initial mass lost. The results confirmed our hypothesis of a shift from predominantly biotic to abiotic control of C and N loss with ongoing litter decay. Microbial and nematode communities regulated litter C and N loss in the early decomposition stages, while soil moisture and legacy effects of initial differences in litter quality played a major role in the late stages of the process. Our analysis, based on statistical associations derived from structural equation modeling, allowed observing and interpreting the complex interactions occurring during the dynamic process of litter decomposition, although ultimate causality could not be established. The joint consideration of the dynamics of litter chemical complexity and the successional trends of decomposer communities under the same framework represents a major advance in understanding the controls over litter decomposition (van der Wal *et al.* 2013; Wickings *et al.* 2012).

Variation of biotic drivers of litter decomposition over time

Our first hypothesis stating that litter decomposition would monotonically increase over time was supported for litter C but not for N loss. After an important loss of both elements between 3 and 7 months of field incubation, there was a slightly continued further loss of C, but not of N, between 7 and 11 months. Higher C and N loss from the high compared to the low quality litter mixtures was found in all but the Sauclieres site, supporting previous large-scale studies and meta-analyses (González & Seastedt 2001; Cornwell *et al.* 2008).

In line with previous studies in forest floors (Schofield *et al.* 1998; Keenan *et al.* 1996), and according to our second hypothesis, litter total phenolic and condensed tannin concentrations decreased rapidly during decomposition. Although the initial concentrations of total phenolics and condensed tannins were four and nine times higher in the low compared to the high litter quality mixtures (Table S1), polyphenol concentrations converged between litter types towards non-significant differences after 11 months of field incubation. These results are in line with the previously observed litter chemical convergence during decomposition (Melillo *et al.* 1989; Parsons *et al*. 2014), but extend it to more recalcitrant secondary metabolites such as tannins (Preston *et al.* 2009).

As hypothesized, along with converging concentrations of polyphenols, we also found converging microbial community level physiological profiles (CLPPs) between contrasting litter types and overall decreasing respiration rates over time. CLPPs were different between the two litter types only after 3 months of field incubation, when the differences in polyphenol concentrations also were still more pronounced. Our results showed little indication for a shift in functional structure, because most of the C substrates, ranging from labile (e.g. glucose) to recalcitrant (e.g. caffeic acid), followed the same pattern (Fig. S2). The overall lower respiration rates after 7 and 11 months of litter field incubation, rather suggest decreased microbial biomass without changes in functional structure. Inversely, nematode communities were similar between litter types after 3 months, and diverged later due to higher abundances of fungal and bacterial feeders in high compared to low quality litter. The quality of litter is important for nematode migration from the soil to the litter, and higher abundance and diversity of nematodes have been observed in high compared to low litter quality (Bjørnlund *et al.* 2005; Szanser *et al.* 2011). In the structural equation models, the concentration of

polyphenols was negatively associated with the abundance of fungal and bacterial feeders, suggesting an inhibitory effect of high polyphenol concentrations. Collectively, our data suggest a close link between the dynamics of plant polyphenols and litter decomposer communities. A promising avenue for future studies would be to combine recent advances in microbial community succession from next-generation sequencing methods (Baldrian & López-Mondejar 2014), with novel high-resolution techniques allowing the identification of qualitative changes of C and N containing molecules (Wickings *et al.* 2012).

Biotic and abiotic drivers of litter C and N dynamics at different decomposition stages

Evaluating the impact of litter decomposition drivers across distinct litter types with different decomposition rates and/or across multiple sites requires studying decomposition at comparable decay stages (Wickings *et al.* 2012; Handa *et al.* 2014; Parsons *et al.* 2014). Using the SEM approach and analyzing particular decay stages, our multi-group comparisons identified which drivers are more important along the litter decomposition continuum. Interestingly, the legacy effect of higher initial litter quality was significantly related to higher C loss only in the two latest stages of decomposition (40-80 and 50-90 % mass loss intervals), unlike when the effects of litter quality were analysed at three arbitrary litter field incubation times (3, 7 and 11 months). No such temporal shift in initial litter quality effects was found for N loss, as the positive effects of litter quality were consistent among decomposition stages. However, there was only very little net N loss after 3 months of litter field incubation, which may indicate similar rates of N immobilization relative to N release (Parton *et al.* 2007).

Approximately 50 % of the total effects of initial litter quality on C loss across decomposition stages were indirectly driven by changing concentrations of polyphenols

and by shifting decomposer communities. It is noteworthy that this was quite different for N, where such indirect effects represented only 25 % of the total litter quality effects. This marked difference between C and N seems to be due to the impact of polyphenols, because the microbial effects along the decomposition process were similar between litter C and N loss. Polyphenols were related to lower litter C loss at early decomposition stages (from 0-40 to 30-70 % mass loss), when high concentrations of tannins in the initial litter may have inhibited decomposition via tannin complexation of microbially produced enzymes (Schimel *et al.* 1998; Coq *et al.* 2010). Such tannin-protein complexes may impair C mineralization more than the access to plant litter-derived proteins and aminoacids, possibly explaining why polyphenols did not appear to have a negative effect on N loss. Interestingly, neither polyphenols nor decomposers mediated the litter quality effects at late decomposition stages. The strong decrease in polyphenol concentrations and converging decomposer communities over time may have reduce their ability to mediate the litter quality impact on late-stage C and N litter dynamics.

Nematodes were influenced by initial litter quality. The abundance of fungal and bacterial feeders was reduced during early decomposition stages (0-40 and 10-50 % mass loss), and then increased in later decomposition stages (40-80 and 50-90 % mass loss) in the high compared to the low litter quality mixtures. However, the effects of litter quality on microbivorous nematode communities were indirectly modulated by polyphenols. The polyphenols-driven litter quality effects on nematodes represented 45 % of the total litter quality effects at the early stages compared to 2 % at later stages. In contrast, litter quality influence on microorganisms was increasingly driven by polyphenols as decomposition progressed: 12 % of the total litter quality effects at the early stages were modulated by polyphenols compared to 41 % at later stages. These results suggest that apparent litter chemical convergence with ongoing decomposition does not imply identical and

predictable effects on decomposer organisms as it was previously concluded (Melillo *et al.* 1989, Preston *et al.* 2009). In addition, litter chemical convergence is the consequence of interacting initial litter chemistry and decomposers communities (Bray *et al.* 2012; Wickings *et al.* 2012), and such interactions may vary depending on the decomposer taxa considered.

Climatic variables are commonly a major driver of decomposition since decomposer activity is regulated by temperature and humidity (Wall *et al.* 2008). Despite the selection of sites along a relatively broad temperature gradient (Table 1, Fig. S1), soil moisture better described climatic variability between sites, and soil temperature accounted for less of the variation in C and N loss than soil moisture. This is probably because soil moisture was the key limiting abiotic factor, and soil temperature and moisture varied independently among our five study sites. Consequently, we did not include soil temperature in the structural equation models, which might have somewhat simplified the contribution of the local climate to decomposition. The positive soil moisture effect on litter C and N loss, and its relative importance compared to other factors, varied over the decay process. At early decomposition stages (0-40 and 10-50 % mass loss) soil moisture had a similar impact on C and N loss like litter quality. In contrast, at late decomposition stages (40-80 and 50-90 % mass loss) soil moisture represented the major influence on litter C and N loss. These soil moisture effects resulted most likely from direct water availability effects on biological processes (Wardle *et al.* 2004), because they were not mediated by changes in polyphenol concentrations or community-level shifts in microbes and nematodes. This distinction of moisture effects is an important result, as most studies addressing the effects of climatic conditions on litter decomposition cannot decouple between direct and indirect effects (Allison *et al.* 2013).

Conclusions

The combined use of polyphenol measurements and community-level assessments of microbes and nematodes through time allowed the establishment of a link between the dynamics of litter chemical complexity and decomposer communities (Wickings *et al.* 2012). Most importantly, the analysis of consistent decay stages along the litter decomposition continuum clearly indicated that the relative control over litter C and N loss by biotic and abiotic factors can change dramatically during the process of decomposition. Along with the incorporation of local-scale spatial variability in control factors (Bradford *et al.* 2015), litter decomposition models should also consider the temporal variation in the importance of such factors. This is critical for the improvement of predictions of litter C and N dynamics, and the assessment of the amount and chemical composition of litter-derived soil organic matter (Grandy & Neff 2008), and its stability under climate change (Crow *et al.* 2009).

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Table 1. Characteristics of the five study sites (in the order of decreasing soil moisture). The soil variables are means ± 1 SE (n = 4). Soil moisture and temperature data are the means along the whole study period (11 months, temporal dynamic in Fig. S1), and were monitored using specific surface soil (5 cm depth) sensors.

Site	Sauclières	Col de Faubel	Mont Aigoual	Lagarde d'Apt	Sainte Baume
Coordinates	43°58' N 3°22'E	44°5'N 3°31'E	44°7'N 3°34'E	43°58'N 5°28'E	43°20'N 5°46'E
Elevation (m a.s.l.)	756	1307	1500	1131	728
Slope (º)	5	8	12	8	5
Soil moisture (%)	28.2	22.7	15.1	13.5	7.8
Soil temperature (ºC)	11.1	8.1	7.2	9.9	12.2
Soil pH	6.9 ± 0.10	4.9 ± 0.04	4.8 ± 0.06	6.1 ± 0.37	7.1 ± 0.05
Soil clay (%)	19.1 ± 0.91	17.3 ± 1.04	17.1 ± 1.78	39.4 ± 2.26	51.8 ± 2.99
Soil silt (%)	16.0 ± 0.64	16.6 ± 0.84	27.0 ± 1.79	39.6 ± 2.25	35.9 ± 2.50
Soil sand (%)	64.9 ± 1.45	66.1 ± 1.86	56.0 ± 3.47	21.1 ± 1.23	12.3 ± 1.07
Soil TOC (g kg^{-1})	32.1 ± 3.14	97.5 ± 1.87	120.8 ± 9.64	84.4 ± 16.34	210.5 ± 11.96
Soil N (g kg^{-1})	1.8 ± 0.26	6.0 ± 0.09	8.1 ± 0.63	4.4 ± 0.90	13.8 ± 0.90
Soil C/N	17.8 ± 0.75	16.2 ± 0.23	14.9 ± 0.22	19.3 ± 0.40	15.3 ± 0.20
Soil NO_3^--N (mg kg^{-1})	3.1 ± 2.92	0.3 ± 0.10	1.9 ± 0.11	1.3 ± 0.62	6.6 ± 0.86
Soil NH_4^+-N (mg kg^{-1})	26.1 ± 13.13	30.4 ± 1.39	38.5 ± 4.69	31.6 ± 7.16	55.5 ± 4.47
Soil Olsen P (mg kg^{-1})	24.0 ± 4.81	44.0 ± 3.19	25.5 ± 2.60	40.5 ± 6.29	57.8 ± 4.19

Figure captions

Figure 1. *A priori* conceptual structural equation model (SEM) depicting pathways by which initial litter quality, soil moisture, polyphenols, microbes and nematodes may influence litter C or N loss (two independent SEM) across sites. This *a priori* model was used for multi-group comparisons along six decomposition stages representing the smoothing groups selected using 40 % mass loss intervals. Single-headed black arrows indicate a hypothesized causal influence of one variable upon another. 'Litter quality' indicates legacy effects of initial differences in quality of the litter mixtures. 'Soil moisture' and 'Polyphenols' are the component 1 from two different PCAs. 'Soil moisture' is positively related with mean soil moisture, and 'Polyphenols' is positively related with the litter concentration of condensed tannins and total phenolics. 'Microbes' and 'Nematodes' are the first axis from the NMDS (see Fig. 4.), with 'Microbes' positively related to the respiration rates of most of the C sources, and 'Nematodes' negatively related with the abundance of bacterial and fungal feeders.

Figure 2. Effects of site, litter quality (high: *A. glutinosa* + *F. angustifolia* and low: *A. glutinosa* + *P. terebinthus*) and litter field incubation time on litter C (A) and N (B) loss. For simplification, only significant ($P < 0.05$) treatments or interactions are shown, and the non-significant ones are collapsed (e.g. there were no differences among sites on C loss). Different letters indicate significant differences between time periods for each site after simple main effects tests. Bars are means ± 1 SE. See Table S2 for statistical analyses.

Figure 3. Effects of site, litter quality (high: *A. glutinosa* + *F. angustifolia* and low: *A. glutinosa* + *P. terebinthus*) and litter field incubation time on the litter concentrations of

total phenolics (A) and condensed tannins (B) along the decomposition process (referred to the values of the initial litter). For simplification, only significant ($P < 0.05$) treatments or interactions are shown. Different letters indicate significant differences between time periods for each site after simple main effects tests. Bars are means ± 1 SE. See Table S3 for statistical analyses.

Figure 4. Effects of site, litter quality (high: *A. glutinosa* + *F. angustifolia* vs. low: *A. glutinosa* + *P. terebinthus*) and litter field incubation time on litter microbial community-level physiological profiles (A) and litter nematode functional group composition (B). For simplification, only significant ($P < 0.05$) treatments or interactions are shown. See Table S4 for statistical analyses. With increasing distance between two treatments, the nematode community and microbial CLPP were more dissimilar. Stress levels = 0.06 in A) and B). Significant Pearson correlations between the NMDS axes and the individual nematode functional groups (A) and C substrates (B) are shown in the boxes, with the arrow representing the sign of the correlation. Values represent means ± 1 SE.

Figure 5. Standardized total effects derived from the multi-group comparisons of SEM evaluating the drivers of litter C and N loss across sites along the decomposition process. The six decomposition stages compared with the multi-group procedure represent the smoothing groups selected using 40 % mass loss intervals. To minimize redundancy among figures, we show one black bar for both the C and N loss SEM when the path coefficient is the same, but differentiate among C (black bars) and N (grey bars) loss when the path coefficients differ. Significant differences in the path coefficients between decomposition stages can be found in Table S5. Goodness-of-fit tests of the multi-group comparisons were: C loss (P value of χ^2 test = 0.04, GFI = 0.950, RMSEA = 0.045), N

loss (P value of χ^2 test = 0.08, GFI = 0.949, RMSEA = 0.030). See Fig. 1 for the model structure proposed in the *a priori* C and N loss SEM, and Materials and Methods section for description of mass loss smoothing groups.

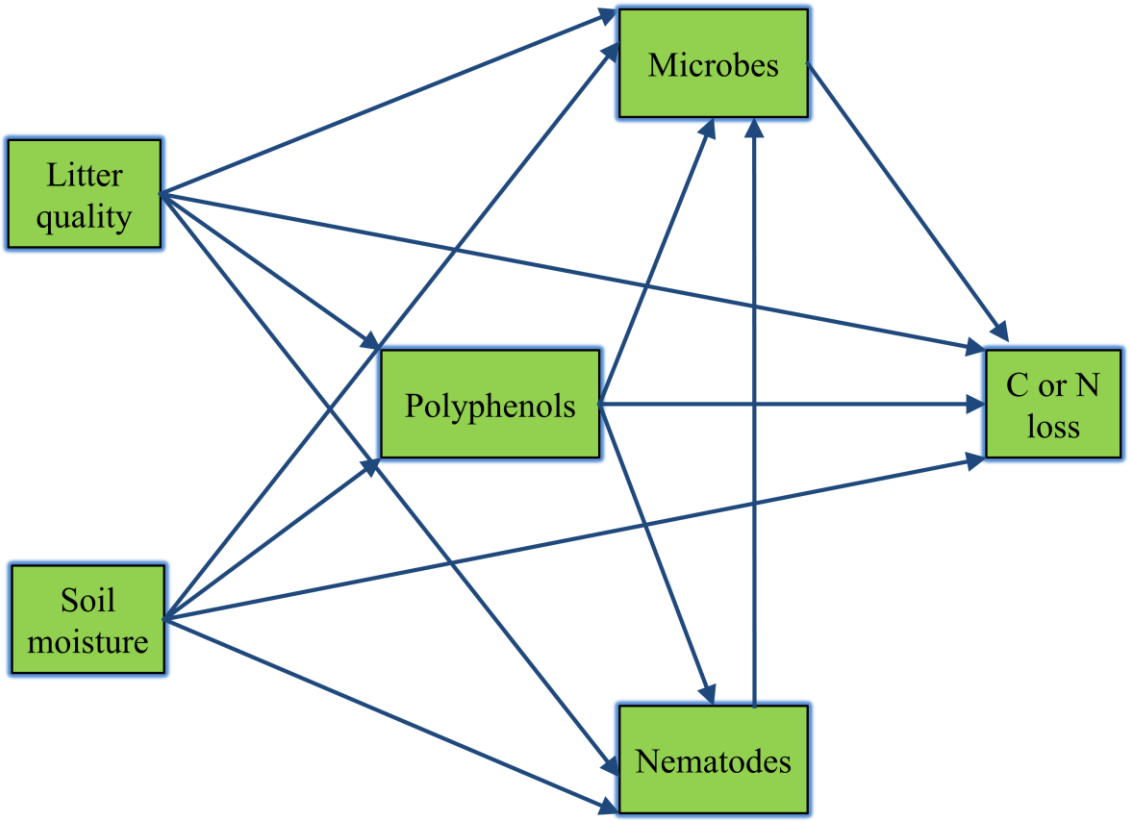

Microbes
Litter quality
Polyphenols
C or N loss
Soil moisture
Nematodes

Figure 1

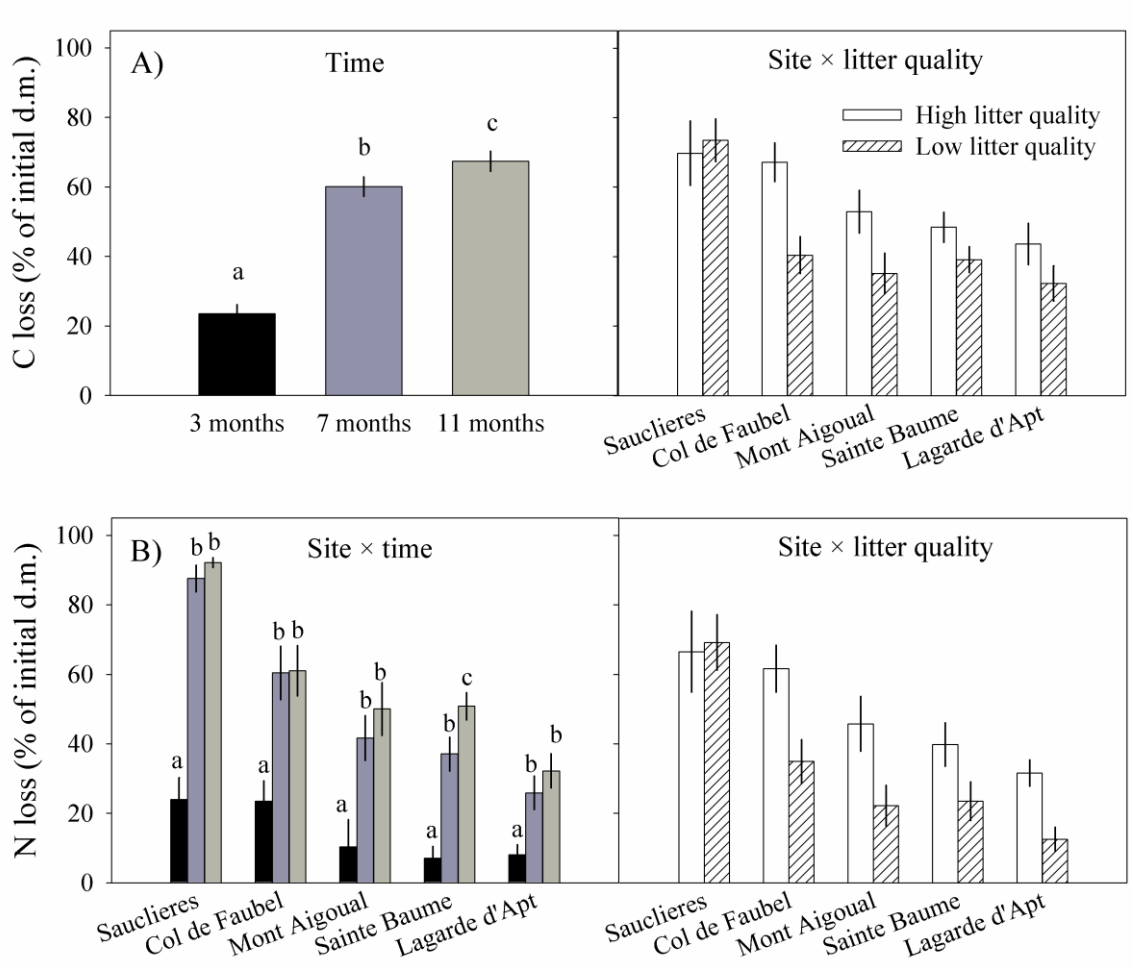

A)
Time
C loss (% of initial d.m.)
100
80
60
40
20
0
a
b
c
3 months
7 months
11 months
Site × litter quality
High litter quality
Low litter quality
Sauclieres
Col de Faubel
Mont Aigoual
Sainte Baume
Lagarde d'Apt
B)
Site × time
N loss (% of initial d.m.)
a
b b
a
b b
a
b
b
a
b
c
a
b
b
Site × litter quality
Sauclieres
Col de Faubel
Mont Aigoual
Sainte Baume
Lagarde d'Apt

Figure 2

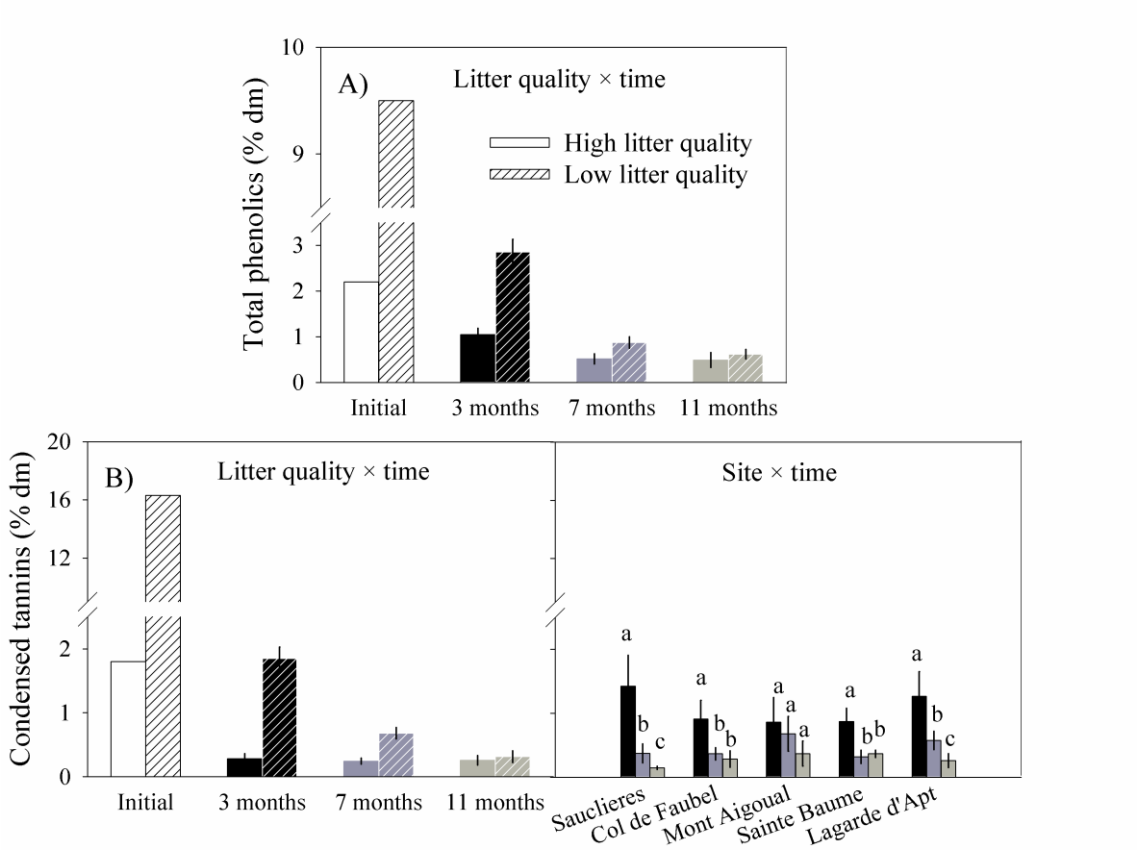
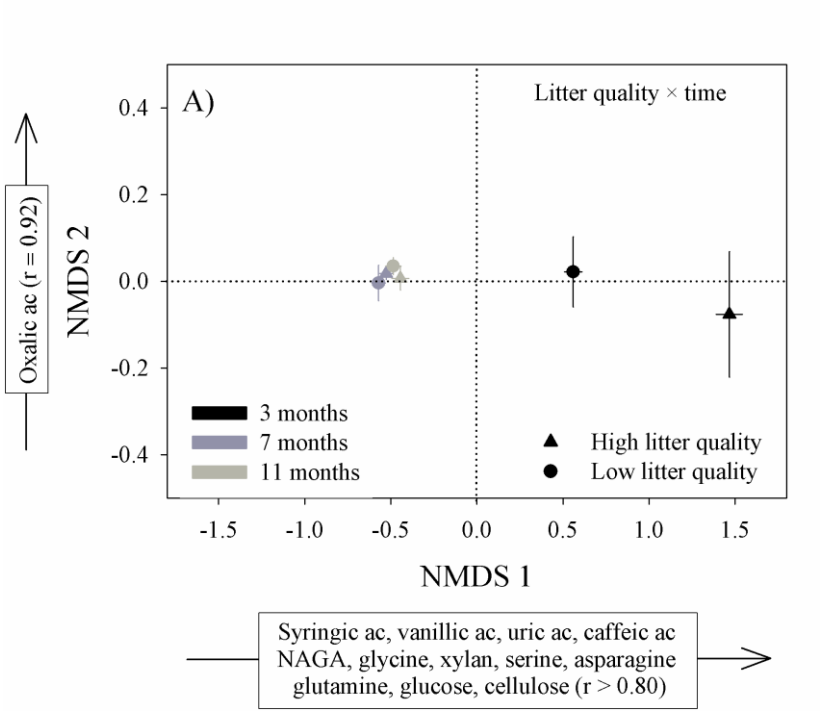
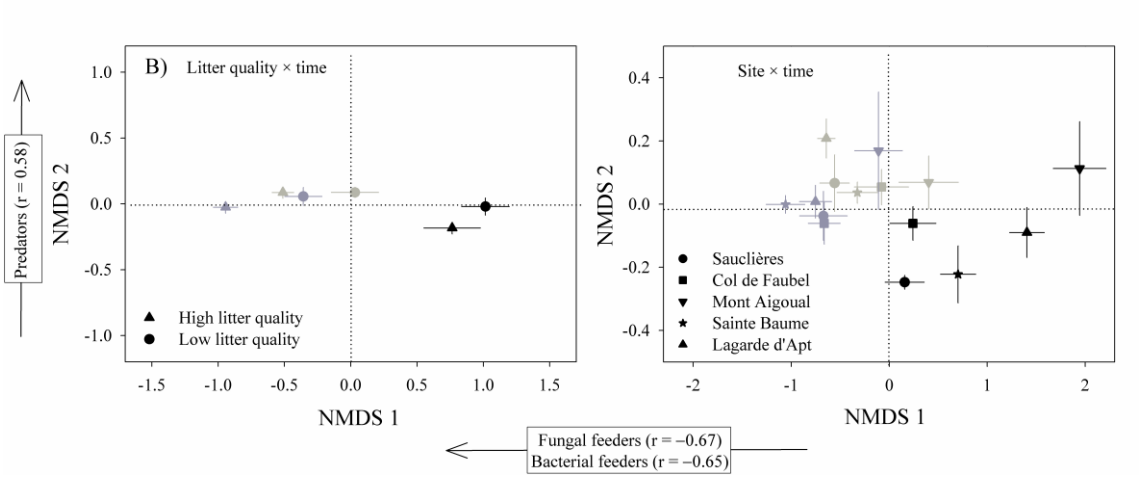

A)
Litter quality × time
High litter quality
Low litter quality
Total phenolics (% dm)
10
9
3
2
1
0
Initial
3 months
7 months
11 months
B)
Litter quality × time
Site × time
Condensed tannins (% dm)
20
16
12
2
1
0
a
b
c
Sauclieres
Col de Faubel
Mont Aigoual
Sainte Baume
Lagarde d'Apt

Figure 3



1



2

3 **Figure 4**

4

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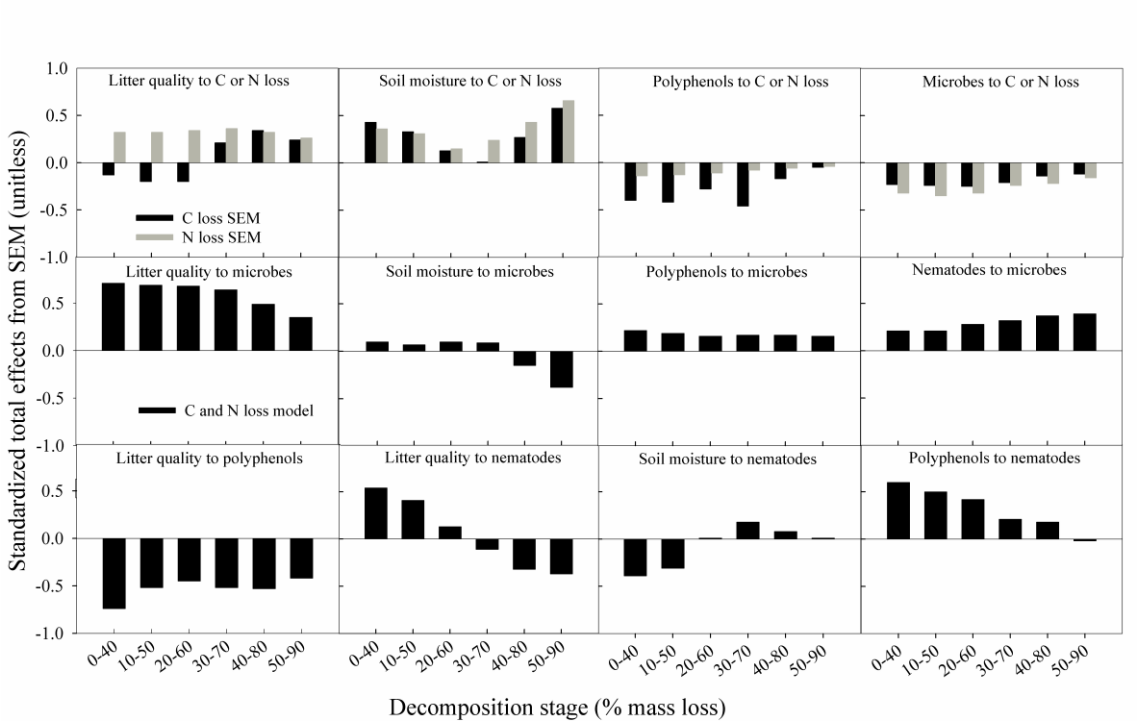
Litter quality to C or N loss
Soil moisture to C or N loss
Polyphenols to C or N loss
Microbes to C or N loss
C loss SEM
N loss SEM
Litter quality to microbes
Soil moisture to microbes
Polyphenols to microbes
Nematodes to microbes
C and N loss model
Litter quality to polyphenols
Litter quality to nematodes
Soil moisture to nematodes
Polyphenols to nematodes
Standardized total effects from SEM (unitless)
1.0
0.5
0.0
-0.5
-1.0
0-40
10-50
20-60
30-70
40-80
50-90
Decomposition stage (% mass loss)

Figure 5