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RESEARCH ARTICLE

Plant litter chemistry drives long-lasting changes in the catabolic capacities of soil microbial communities

Malo Y. Bourget[1] | Nicolas Fanin[2] | Nathalie Fromin[3] | Stephan Hättenschwiler[3] | Catherine Roumet[3] | Ammar Shihan[3] | Raoul Huys[1] | Marie Sauvadet[4] | Grégoire T. Freschet[1]

[1]Station d'Écologie Théorique et Expérimentale, CNRS, Moulis, France

[2]INRAE, Bordeaux Sciences Agro, UMR 1391 ISPA, Villenave-d'Ornon, France

[3]Centre d'Ecologie Fonctionnelle et Evolutive, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France

[4]Eco&Sols, Univ Montpellier, IRD, INRA, CIRAD, Montpellier SupAgro, Montpellier, France

Correspondence
Grégoire T. Freschet
Email: gregoire.freschet@sete.cnrs.fr

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Abstract

1. Although microbial communities play an important role in explaining plant litter decomposition rates, whether and how litter chemistry may alter catabolic capacities of soil microbial communities remains poorly studied.
2. During a 1-year litter decomposition experiment of 12 herbaceous species with contrasting litter chemistry, we examined the effect of plant litter type (roots vs. leaves) and litter chemical traits on the resulting capacity of soil microbial communities to degrade a wide range of carbon substrates of variable complexity (MicroResp™ method).
3. Litter chemistry impacted both the total catabolic activity as well as specific catabolic capacities of microbial communities. In the early stages of litter decomposition total catabolic activity was mainly influenced by the amount of C and N in litter leachates, and litter N, P and Mg, then, later, by lignin concentrations. Some specific catabolic capacities could also be related to litter initial chemistry. Overall, litter trait effects on soil microbial communities decreased over time and the relative importance of traits shifted during the decomposition process.
4. Our results highlight that litter chemistry is a strong driver of catabolic capacities of microbial decomposers and, while its effect fades with time, it remains substantial throughout the litter decomposition process. These long-lasting effects of litter chemistry suggest a persistent control on microbial catabolic capacities in ecosystems with recurrent litter production. Soil microbial catabolic activities were driven by broadly the same chemical traits across leaf and root litters.
5. *Synthesis*. Such long-lasting effects of litter chemistry on catabolic capacities of microbial communities may represent a substantial indirect driver of the decomposition process. Disentangling the relative importance of this overlooked effect of litter chemistry on decomposition represents the next challenge. We argue that such research line should open ground-breaking perspectives for reconsidering our current understanding of the mechanistic links between litter traits and decomposition rate.

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KEYWORDS
leaf litter, legacy effects, litter decomposition, litter traits, microbial catabolic capacities, root litter, soil microbial communities

1 | INTRODUCTION

Litter decomposition is a fundamental process influencing carbon (C) and nutrient cycling in terrestrial ecosystems (Swift et al., 1979). It has long been thought that this process was mainly controlled by temperature and humidity at large spatial scales (Coûteaux et al., 1995). However, an increasing body of literature has suggested that microclimate and the interactions between decomposers and litter quality are also important drivers controlling litter decomposition at the local scale (Bradford et al., 2016, 2017; Joly et al., 2017; Wickings et al., 2012). These results underline the need to better consider microbial communities and their activity at the scales at which they perform decomposition (Fanin et al., 2019) to get a better understanding of litter decomposition. Shifts in the composition of soil microbial communities may have important consequences on litter decomposition (Voříšková & Baldrian, 2013).

It has been proposed on multiple occasions that changes in microbial activity can be relatively well predicted by changes in litter traits across various spatiotemporal scales (Cleveland et al., 2014; Fanin & Bertrand, 2016; Šnajdr et al., 2011). Soil microbial communities, including bacterial and fungal communities, are often primarily limited by organic C inputs and access to energy (Hättenschwiler et al., 2011; Soong et al., 2020). Because leaves display high contents in soluble compounds, cellulose and hemicellulose, they generally decompose faster than roots and stems, which are more recalcitrant and enriched in lignin (Freschet et al., 2013; Lin et al., 2020). Such a variability in litter chemistry may, in turn, affect the structure of microbial decomposer communities due to contrasting affinities for different C substrates (Martínez-García et al., 2018; Sauvadet et al., 2019). Generally, copiotroph (fast-growing) organisms typically thrive in the presence of easily degradable C sources, whereas oligotrophs exhibit slower growth rates and are likely to outcompete copiotrophs in conditions of low resource availability (Fanin & Bertrand, 2016; Fierer et al., 2007; Ho et al., 2017). Most soil microorganisms are able to degrade simple carbohydrates or holocellulose. However, the degradation of recalcitrant C compounds such as lignin or secondary metabolites requires the synthesis of oxidative enzymes such as peroxidase or phenol oxidase (Thevenot et al., 2010). As such, recalcitrant litters should stimulate the synthesis of oxidative enzymes and accelerate the degradation of complex molecules (Sauvadet, Chauvat, Cluzeau, et al., 2016). Microbial communities can also be limited by nutrients such as nitrogen (N) and phosphorus (P), in soils where C is present in non-limiting conditions (Fanin et al., 2016). As a consequence, organic matter chemical composition often selects for organisms adapted to degrade it (Ayres et al., 2009; Freschet et al., 2012b; Strickland et al., 2009), but whether and how the functional abilities of microbial communities reflect the biochemistry of organic matter inputs need further investigation.

Changes in litter quality over the course of the decomposition process may also have repercussions on the fate of various C forms within the litter (Pascault, Nicolardot, et al., 2010; Wickings et al., 2012). For instance, while the amount of C and N in litter leachates is a primary factor controlling microbial activity at the early stages of decomposition (Joly et al., 2016; Pascault, Cécillon, et al., 2010), lignin is one of the main drivers affecting enzyme production at later stages (Lashermes et al., 2016). Yet, how catabolic capacities of microbial communities vary along the course of the litter degradation and change in chemical composition is still an open question. Initial litter chemistry, and particularly the contrast between litter and soil organic matter composition may further influence the duration of litter influence on soil microbial catabolic activities (as seen between leaf and root litter; Sauvadet et al., 2019). Generally, it remains unclear whether these effects are transient or persist at the end of litter degradation.

In this study, our main objective was to assess the catabolic capacities of microbial communities in soil mixed with litter and how they vary under the influence of litter chemical traits as the decomposition proceeds. To do so, we incubated leaves and roots of 12 herbaceous species in soil microcosms under favourable decomposition conditions for 367 days. We quantified the temporal changes in the ability of soil microbial communities to respire on a wide range of C substrates via the Microresp™ method and evaluated which litter traits were the best predictors of these catabolic activities over time. We hypothesized that (i) initial litter chemistry should control the catabolic capacities of microbial communities at all stages of the decomposition process. Specifically, we predicted that simple C compounds and nutrients should be the main litter traits influencing catabolic capacities during the early stages, while lignin and tannins should be the most influential litter traits during the later stages. We further expected that (ii) differences in catabolic capacities along the litter decomposition process should depend on the type of litter (e.g. roots vs. leaves) because of important differences in initial litter quality. Specifically, we expected that roots should stimulate the use of complex carbon compounds (e.g., phenolic acids) more than leaves, which should stimulate more the use of simple carbon compounds (e.g., carbohydrates). Finally, we hypothesized that (iii) litter-driven differences in catabolic capacities should decrease in later decomposition stages as litter quantity decreases and litter-derived compounds are further transformed by soil decomposers, so that the catabolic capacities of microbial communities converge towards that of pure soil communities (no litter treatment).

2 | MATERIAL AND METHODS

2.1 | Production of plant material

Twelve herbaceous species, representative of Southern France Mediterranean old-field succession, were selected based on contrasting above- and below-ground chemical traits (i.e. tissue quality), life histories, and taxonomic groups (Table 1; Birouste et al., 2012). Seeds from these species were collected from naturally occurring populations in the vicinity of Montpellier (43°36′N; 3°52′E), Southern France, in August 2014 and set to germinate in September 2014 for 3 weeks. Monocultures of each species were established by transplantation of seedlings to large pots at a plant density of 200 plants m^{-2}. Pots were filled with a naturally decarbonated soil (absence of $CaCO_3$ allowed measurements of litter C isotopes, as detailed in a companion study conducted on the same experiment; Huys et al., 2022) sieved at 5 mm with a sandy loam texture (9% clay, 26% silt, 65% sand), a pH of 7 and relatively low organic C and N concentrations (15 and 1.1 g kg^{-1}, respectively), P availability (0.035 g kg^{-1} Olsen-P) and cation exchange capacity (7.75 cmol kg^{-1}). The soil, excavated in Villefort (France; 44°43′N, 3°92′E), was a brunisol developed on a schist parent rock. Plants were grown for 5 months in a greenhouse, under natural light conditions and temperature allowed to fluctuate between 15 and 28°C. To ensure plant growth, soils were subjected to monthly addition of N solution (50/50 nitrate/ammonium in the form of $Ca[NO_3]_2Na$ and NH_4Cl) in increasing amount from 2 to 8 g N m^{-2} for a total of 18 g N m^{-2}. During the last month before harvest, plant watering was progressively reduced and stopped to reproduce typical conditions of Mediterranean climate summer drought and induce plant senescence. All plant species were still at a vegetative stage. At the end of the senescence period, plants were harvested and sorted into (dead) leaf and fine-root materials (all roots except tap roots corresponding to roots of the three most distal orders; Freschet & Roumet, 2017). Root samples were carefully cleaned with water before sorting. All material was air-dried at 25–30°C. Only leaf litter and fine-root litter were subsequently used in trait analyses and decomposition experiments.

2.2 | Litter trait measurements

A set of 12 chemical traits was measured on leaf and fine root litter subsamples for the 12 species. This included the concentrations of six elements, carbon (C), nitrogen (N), phosphorus (P), magnesium (Mg), calcium (Ca) and manganese (Mn), the concentrations in water-soluble C compounds, hemicellulose, cellulose, lignin and tannins, and the concentrations of soluble C, N and P in litter leachates. C and N were measured on a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd.). Phosphorus, Mg, Ca and Mn were measured, after acid mineralization, by plasma emission spectrometry (ICP-MS, Thermo Scientific iCAP Q, Thermo Fisher Scientific GmbH, Germany). The concentrations of water-soluble compounds, hemicellulose, cellulose and lignin were obtained with the van Soest method (Van Soest, 1963) using a Fibersac 24 fibre analyser (Ankom). Condensed tannins were measured according to the acid butanol method (Waterman & Mole, 1994).

Soluble C, N and P were determined on litter leachates obtained by extracting 0.5 g of litter (air-dried and cut into 1 cm long pieces) in 30 mL of distilled water for 30 min on an end-over-end shaker (20 rpm at 20°C), and then filtered at 0.45 μm. Total organic C and N concentrations in the extracts were analysed using an automated TOC-TN analyser (Shimadzu, TOC-Vcph, Japan). Total P concentration was determined colorimetrically after digestion with sulfuric acid and hydrogen peroxide (35 min at 100°C and 2 h at 360°C) by the molybdenum blue method (Grimshaw et al., 1989), with an autoanalyser (Evolution II; Alliance Instrument).

2.3 | Litter addition experiment

The soil used for litter addition was of the same origin as the soil used for growing the plants and described above. The soil was sieved at 5 mm, spread in trays and humidified to induce seed bank germination. After 2 weeks, the germinated seeds were removed, and the soil was air-dried until constant mass, then sieved at 2 mm and homogenized. Sets of subsamples were used to estimate soil residual humidity (oven-dried at 105°C, soil water content of 0.014 g g^{-1}) and soil field capacity (0.396 g g^{-1}). Leaf litter and fine-root litter from each of the 12 species were cut in 1 cm^2 and 1 cm long pieces respectively, homogenized and 12 samples of 0.4 g were weighed for each of the 24 litter materials. Each of these samples was then thoroughly mixed with 50 g of soil and transferred to a 80 mL plastic flask (hereafter 'microcosm'). Fifteen microcosms of bare soil were prepared in the same way. This resulted in 303 microcosms, representing three replicates of four sequential sampling for the 24 types of soil-litter mixture plus 15 replicates of bare soil (12 species × 2 plant organs × 4 sampling dates × 3 replicates + 15 controls). At the start of the experiment, soils were brought to 80% of field capacity using distilled water, closed with pierced lids allowing gas exchanges and incubated at 25°C in the dark in climatic chambers. To follow litter decomposition dynamics and soil microbial communities' catabolic activities, three replicates of each type of treatment were sampled at four time steps, that is, at early (10 and 38 days), intermediate (157 days) and late (367 days) stages. At time of sampling, microcosm content was spread on plates to dry at 25°C in a well-ventilated room. At each time of sampling, litter decomposition was assessed on a 10 g subsample of incubated soil-litter mixture by sorting manually litter pieces remaining in the soil. Litter samples were ground with a ball mill and measured for C and N concentrations using an elemental analyser (CHN model EA1108; Carlo Erba Instruments). Soil mineral contamination of litter was corrected for by considering litter C concentration rather than mass. More than 90% decomposition was achieved for all 24 litters after 367 days of incubation, with an average of 97% decomposition of the initial litter C (Figure S1).

TABLE 1 List of species and the chemistry of their litter, as represented by the mean concentration of a range of compounds. For each chemical trait, significant differences between root and leaf litter mean traits are indicated by ** ($p < 0.01$) or *** ($p < 0.001$).

Species	Family	Plant functional type	Litter type	Hemicellulose ($mg\,g^{-1}$)	Cellulose ($mg\,g^{-1}$)	Lignin ($mg\,g^{-1}$)	Solubles ($mg\,g^{-1}$)	Tannins (%)
Bituminaria bituminosa	Fabaceae	Legume	Leaf	180.1	146.7	77.3	595.8	0.46
			Root	282.2	206.0	106.5	405.3	0.12
Brachypodium phoenicoides	Poaceae	Graminoid	Leaf	350.1	289.0	41.7	319.2	0.55
			Root	328.0	301.4	119.5	251.1	0.19
Bromus erectus	Poaceae	Graminoid	Leaf	258.9	284.0	31.7	425.4	0.35
			Root	360.3	301.2	102.8	235.7	0.06
Bromus madritensis	Poaceae	Graminoid	Leaf	273.8	249.4	32.7	444.1	0.45
			Root	404.4	293.3	81.9	220.4	0.06
Clinopodium nepeta	Lamiaceae	Forb	Leaf	127.5	94.9	76.9	700.8	0.47
			Root	211.4	240.4	189.5	358.8	0.06
Crepis foetida	Asteraceae	Forb	Leaf	168.0	120.0	96.4	615.6	0.39
			Root	208.9	190.6	172.7	427.8	0.18
Dactylis glomerata	Poaceae	Graminoid	Leaf	270.1	267.0	36.6	426.4	0.47
			Root	352.5	296.1	110.3	241.2	0.18
Daucus carota	Apiaceae	Forb	Leaf	233.8	153.7	64.3	548.2	0.54
			Root	212.8	197.5	154.2	435.4	0.07
Medicago minima	Fabaceae	Legume	Leaf	243.0	170.7	56.4	529.9	0.30
			Root	250.7	224.6	130.4	394.2	0.12
Picris hieracioides	Asteraceae	Forb	Leaf	178.1	160.6	43.1	618.2	0.72
			Root	179.4	193.4	154.8	472.3	0.30
Tordylium maximum	Apiaceae	Forb	Leaf	113.6	159.5	38.5	688.4	0.27
			Root	252.6	214.8	130.3	402.3	0.07
Trifolium angustifolium	Fabaceae	Legume	Leaf	239.0	172.8	44.8	543.4	0.51
			Root	268.4	242.6	136.5	352.6	0.03
Root vs. Leaf difference				**	***	***	***	***

2.4 | Community-level catabolic profiles

The community-level catabolic profile (CLCP, also sometimes referred to as community-level physiological profile, or multiple substrate-induced respiration [SIR]) of the soil microbial community was assessed on approximately 22 g of air-dried soil from sampled microcosms using the MicroResp™ system (Macaulay Scientific Consulting; Campbell et al., 2008). This method is used to estimate the soil microbial functional capacity in C cycling (Hernández-Cáceres et al., 2022) and can generate estimates of both soil total catabolic activity and diversity of soil microbial communities (Bending et al., 2002; Fromin et al., 2020). These soil sub-samples were homogenized and checked to ensure an even distribution of remaining litter fragments within MicroResp DeepWell Microplates. About 0.45 g of air-dried soil including remaining litter fragments was incubated in (analytical) triplicate, in 96-DeepWell Microplates (Fisher Scientific E39199) together with a solution containing 1.5 mg C of a carbon substrate (except for the low-soluble phenolic acids and cellulose for which 0.75 mg C g^{-1} soil was added), so as to reach 80% of field capacity. Carbon substrates included three carbohydrates (D-glucose, xylan, cellulose), one amine (N-acetyl-glucosamine), five amino acids (L-asparagine, L-glutamine, L-lysine, L-serine, L-glycine), three carboxylic acids (malic acid, oxalic acid, uric acid) and three phenolic acids (caffeic acid, syringic acid, and vanillic acid). Gel detection plates were prepared as recommended by the manufacturer with 1% Oxoid Agar, 12.5 μg mL^{-1} Cresol red, 150 mM KCl and 2.5 mM $NaHCO_3$. After an initial 2 h pre-incubation step at 25°C in the dark to account for the lag period, each microplate was covered with a detection plate using a silicone gasket (MicroResp™). The assembly was secured with a clamp and incubated for four additional hours. Optical density at 590 nm (OD) was measured in detection wells before and after incubation using a Victor 1420 Multilabel Counter (Perkin Elmer). Final OD were normalized using pre-incubation OD and converted to SIR rates expressed in μg C-CO_2 g^{-1} air-dried soil h^{-1}. The mean values for the triplicate wells were used to calculate the specific catabolic activity (SIR_i) for each carbon substrate. An estimate of total catabolic activity (SIR_{tot}) was calculated as the sum of all 15 SIR_i rates. This measure of soil SIR is established based on a wide range of C-based substrates, rather than

N (%)	C (%)	P (%)	C leachate (mg g^{-1})	N leachate (mg g^{-1})	P leachate (mg g^{-1})	Mg (mg g^{-1})	Ca (mg g^{-1})	Mn (mg g^{-1})
3.01	41.3	0.14	102.2	7.78	0.63	13.30	36.5	0.10
3.29	37.7	0.27	38.3	4.71	1.03	2.69	13.6	0.10
2.19	43.2	0.10	43.2	6.21	0.52	3.18	5.9	0.14
1.66	43.5	0.15	25.9	4.39	0.89	1.33	5.3	0.31
2.96	41.2	0.16	68.9	10.58	0.93	4.73	8.0	0.13
1.91	41.9	0.19	26.4	3.83	0.40	1.07	4.4	0.18
3.44	41.0	0.20	50.1	10.88	1.03	5.39	17.7	0.18
1.74	40.7	0.10	19.4	2.89	0.90	1.66	5.8	0.23
3.66	43.0	0.19	96.5	8.20	0.84	8.13	21.9	0.10
2.17	42.2	0.15	29.7	2.04	0.41	4.34	11.8	0.17
3.53	37.9	0.25	78.8	11.50	1.21	14.34	31.0	0.09
2.69	40.0	0.31	33.6	3.70	1.11	3.67	14.5	0.18
2.85	41.0	0.13	69.5	10.36	0.68	5.35	8.6	0.17
1.45	43.3	0.11	20.5	2.52	0.55	0.99	4.4	0.15
2.56	39.8	0.13	84.7	8.06	0.59	12.96	38.7	0.10
2.19	40.9	0.15	57.1	5.80	0.77	3.51	10.3	0.13
3.39	40.1	0.17	109.7	10.17	0.76	5.59	36.7	0.12
3.00	38.9	0.25	30.7	3.82	0.85	3.54	13.9	0.37
3.70	38.5	0.36	79.7	10.58	1.23	13.28	31.9	0.18
2.63	41.3	0.18	42.9	4.87	0.78	4.07	12.1	0.40
4.18	36.2	0.36	104.8	12.24	0.72	12.07	52.2	0.19
2.51	34.1	0.31	23.7	3.41	0.92	4.37	17.1	0.64
3.17	40.3	0.20	116.6	7.11	1.09	7.00	43.9	0.12
3.00	39.1	0.30	17.7	2.18	0.69	3.15	11.2	0.17
***			***	***		***	***	**

based on glucose addition only, and represents therefore the capacity of soil microbial communities to use heterogenous C-sources to stimulate soil organic matter decomposition (i.e. the mineralization of organic C into carbon dioxide). For each sample, SIR_i rates were converted into relative substrate induced respiration ($rSIR_i$; Equation 1), to assess which catabolic capacities were either higher or lower than the overall mean within a sample.

$$rSIR_i = \frac{SIR_i}{SIR_{tot} / 15} - 1 \quad (1)$$

A Shannon catabolic diversity index estimating the degree of evenness among all 15 catabolic activities tested was calculated (Equation 2).

$$H' = \sum_{i=1}^{15} SIR_i \times \ln(SIR_i) \quad (2)$$

Low H' values mean that a reduced set of catabolic capacities drive microbial total catabolic activity, while high values mean that a high number of catabolic capacities contribute fairly equally to microbial total catabolic activity.

2.5 | Statistical analyses

Statistical analyses were performed with the R software (R Core Team, 2020). To assess the impact of litter chemical traits on microbial communities' SIR, SIR_{tot} and H' at each sampling time, a model averaging approach was used (MuMIn package; Barton, 2020). This procedure was repeated for all 24 litters together, as well as for each of the 12 leaf litters and root litters taken separately. As litter initial chemical traits were collinear, models were computed according to a correlation matrix (Table S1), which precluded highly correlated variables ($r > 0.7$) to be incorporated in the same model (see Dormann et al., 2013). Predictors were chosen based on reported effects in the literature on all our dependent variables; in particular, microbial activity is sensitive to the quality of the fresh organic matter added (e.g. hemicellulose and cellulose, lignin, tannins and C leachates; Soong et al., 2020) and can also be modulated by soil nutrient availability, including N, P, Ca, Mn and Mg (e.g. Fanin et al., 2016). To limit the number of model parameters, ratios and interactions between chemical traits were also omitted. For the separate leaf and root analyses,

Ca and soluble concentrations were further omitted, due to their lack of predictive value and to avoid model over-parametrisation. As litter traits are typically measured on different numerical scales, they were standardized prior to model computation to enable interpretation of parameter estimates following model averaging (Grueber et al., 2011). Then, models were computed with the *dredge* function according to this correlation matrix. Estimates from models with $\Delta AIC_C < 2$ were then averaged (*model.averaging* function) with the zero method (Galipaud et al., 2017). Following model averaging, averaged estimates were used to compute predicted values for each microbial community characteristic. These predicted values were then regressed on observed ones. Paired Mann–Whitney tests were then conducted on corresponding residuals to assess if litter type (i.e. root vs. leaf) could explain additional variance.

To assess the effect of species, plant functional type (legume, forb or graminoid), time (10, 38, 157 or 367 days) and litter type (control, leaf litter or root litter) on SIR_{tot} and H', two-way ANOVAs were performed (CAR package; Fox & Weisberg, 2019). Prior to the ANOVAs, SIR_{tot} and H' were log-transformed to meet parametric assumptions. ANOVAs were followed by post-hoc tests (EMMEANS package, Lenth, 2020). To assess the effect of time and litter type on specific microbial catabolic capacities, we used a different approach. We used dissimilarity between catabolic profiles of microbial communities—based on their $rSIR_i$—using Bray–Curtis distance matrices. Then, effects of litter type and time were investigated using permutational multivariate ANOVA (PERMANOVA, VEGAN package; Oksanen et al., 2020). PERMANOVA was followed by a pairwise multilevel comparison (PAIRWISEADONIS package; Martinez Arbizu, 2017) and a similarity percentage (SIMPER, VEGAN package; Oksanen et al., 2020) procedure to identify differences between groups and contribution of catabolic capacities to these dissimilarities among those groups (Clarke, 1993). Additionally, we computed a non-metrical multidimensional scaling (NMDS) with vector fitting. Vector fitting is based on multiple linear regression of variables of interest (i.e. catabolic capacities of microbial communities) on coordinates of the first two principal axes of the NMDS.

3 | RESULTS

3.1 | Effect of species, plant functional type and initial litter traits on soil microbial community functions along decomposition

Species identity had no effect on total catabolic activity (SIR_{tot}) of the soil microbial communities during the decomposition process but significantly affected their catabolic evenness (H'; $p < 0.01$) and relative changes in specific catabolic capacities ($p < 0.001$; Tables S2 and S3). Plant functional type (legume, forb or graminoid) had no effect on SIR_{tot}, H' and relative changes in specific catabolic capacities (Tables S4 and S5).

Several chemical traits of the initial litter material significantly influenced SIR_{tot}, H' and relative changes in specific catabolic capacities. However, SIR_{tot}, H' and relative changes in specific catabolic capacities were influenced by different sets of traits throughout the decomposition process (Figure 1; Figure S2). The set of traits driving SIR_{tot} was only partly consistent when tested on all 24 litters or on the 12 root and leaf litters separately (Figure 1). Only detailed relationships across the full gradient of litter chemistry (all leaf and root litter considered together) are presented below.

The SIR_{tot} was positively related to initial litter N concentration at the beginning of decomposition (day 10), then to N concentration in litter leachates (day 38) and, at last, to C concentration in litter leachates and to litter lignin concentration (day 157). Additionally, SIR_{tot} was negatively related to the initial C concentration in litter leachates and to litter P concentration (day 10) and subsequently to the litter Mg concentration (day 38). At the end of the decomposition process, when an average of 3% of initial litter C remained as retrievable fragments (day 367), no litter chemical trait had a substantial influence on SIR_{tot} (Figure 1a).

While catabolic evenness was only influenced by the litter P and Mn concentrations at day 157 (Figure 1b), several litter traits influenced the specific catabolic capacities of microbial communities throughout the decomposition process (Figure S2).

Out of the 15 catabolic capacities (i.e. rSIR) investigated, litter chemistry influenced only a limited number at each sampling time (4 at day 10, 3 at day 38, 5 at day 157 and 4 at day 367). These were not the same at each sampling time and overall there were 10 out of 15 catabolic capacities significantly affected at some point during the entire decomposition process (Figure S2). Particularly, litter N, leachates N and Mn concentrations were positively related to glucose and cellulose mineralization at day 38 or 157, while the litter Ca concentration showed contrasting effects over time (positive at day 157 and negative at day 367, Figure S2). Capacities of microbial communities to degrade amino acids were significantly related to the litter Mg concentration at day 10 (positively for serine and negatively for lysine) and to litter Mn concentration at day 157 (positive for lysine and glycine) (Figure S2). Malic acid degradation was the activity that could be linked with litter chemical traits almost all along the whole decomposition process (at the exception of day 38), with, among others, a positive influence of litter Mn concentration at both day 10 and day 367 (Figure S2). Catabolic capacities of microbial communities to degrade phenolic acids were explained by initial litter chemistry at different decomposition steps. While vanillic acid degradation was related to the amount of cellulose and N in litter leachates at day 38, caffeic acid was related to the litter lignin concentration at day 157 and syringic acid was related to the litter hemicellulose concentration at day 367 (Figure S2). Also, litter P concentration did not explain any of the microbial catabolic capacities measured here.

3.2 | Effect of litter type on soil microbial community metabolism

During litter decomposition, SIR_{tot} of soil mixed with litter was significantly higher than that of soil incubated without litter material (Table 2a; Figure 2a). However, no significant differences could

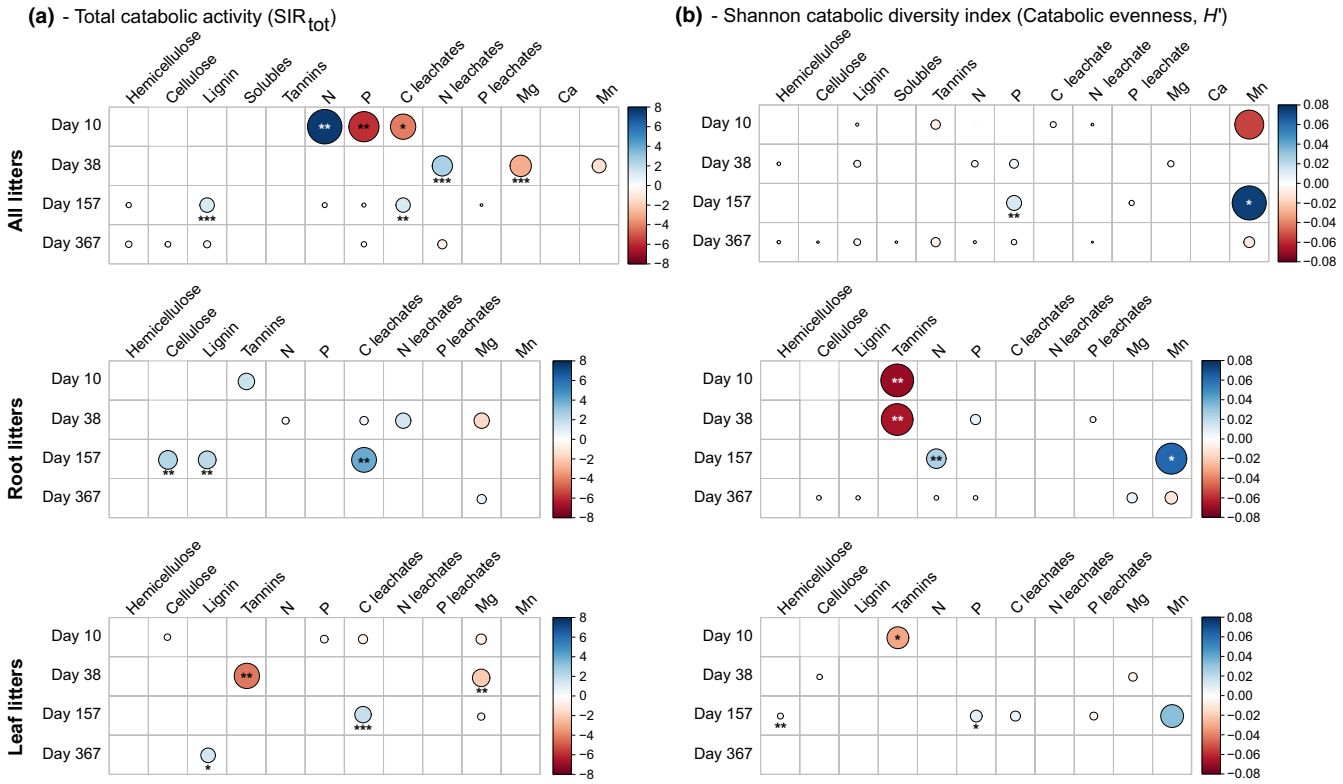

FIGURE 1 Heatmaps of the estimated effects of litter initial traits on microbial (a) total catabolic activity and (b) Shannon catabolic diversity index at the different sampling times. Root and leaf litters were analysed together (upper panels) and separately (lower panels). Estimates were obtained through a model averaging procedure (*zero* method) from models with ΔAICc < 2. Circle size and colour correspond to coefficient estimates values. Circles with seemingly no colour represent coefficient estimates close to zero. Empty squares correspond to initial traits that were not retained in models with $\Delta AIC_C < 2$. *, ** and *** show significance levels at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

TABLE 2 Main effects of (a) litter treatment (control without litter, leaf or root litter) and (b) sampling time (10, 38, 157 or 367 days) on total catabolic activity and catabolic evenness (estimated by Shannon catabolic diversity index) of soil microbial communities as revealed by post-hoc comparison following two-way ANOVAS. †, *, ** and *** show significance levels at $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

	Total catabolic activity			Catabolic evenness		
	df	t	p	df	t	p
(a)						
Leaf vs. Root	96	0.251		96	−0.144	
Control vs. Leaf	96	−11.642	***	96	4.367	***
Control vs. Root	96	−11.483	***	96	4.276	***
(b)						
10 vs. 38 days	96	5.948	***	96	1.742	
38 vs. 157 days	96	4.122	***	96	−1.064	
157 vs. 367 days	96	0.57		96	−2.39	†
10 vs. 157 days	96	10.07	***	96	0.678	
10 vs. 367 days	96	10.64	***	96	−1.712	
38 vs. 367 days	96	4.692	***	96	−3.454	**

be observed between soils amended with either leaf or root litter. Catabolic evenness was significantly lower in soils mixed with litter than in pure soil. However, there were no differences in SIR_{tot} nor in catabolic evenness between soils mixed with leaf and those mixed with root litter (Table 2a; Figure 2b).

The catabolic profiles of microbial communities (estimated with distance matrices) differed between pure soil and soil mixed with litter (either roots or leaves) at all sampling times (Figure 3; Table 3). Dissimilarities between pure soil and soil mixed with litter were mainly driven by catabolic capacities of microbial communities to degrade amino acids, carboxylic acids and phenolic acids, but not carbohydrates (Table 3). Additionally, catabolic profiles of microbial communities mixed with either root or leaf litter were significantly dissimilar at day 10 and day 157, but not at day 38 and 367 (Table 3).

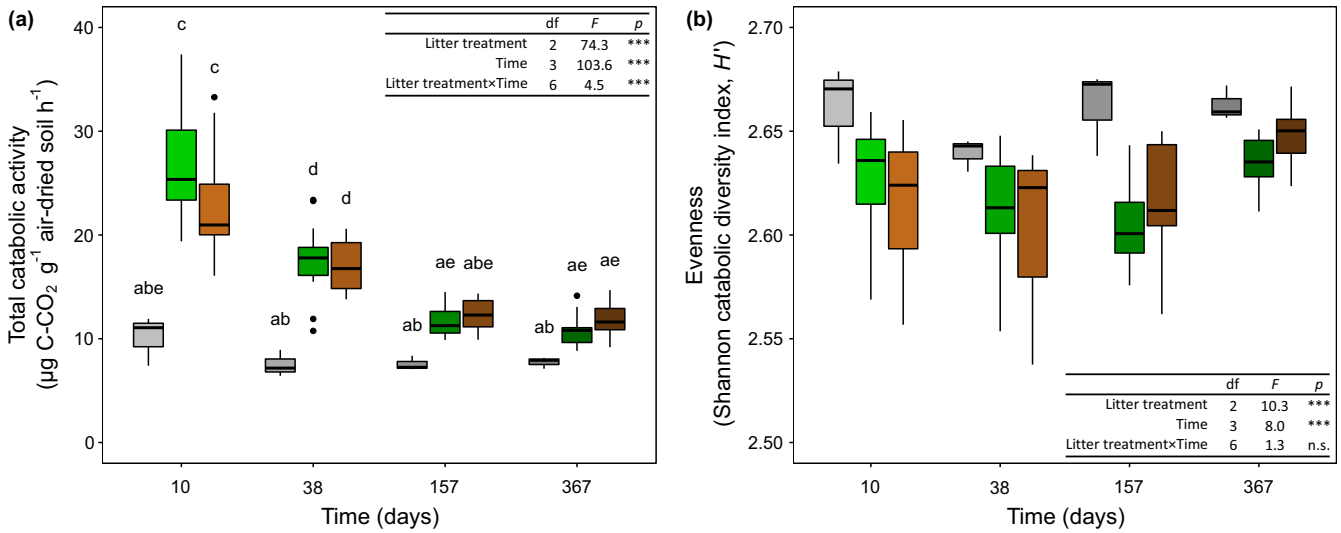

FIGURE 2 Evolution of microbial communities (a) total catabolic activity and (b) catabolic evenness in control soil (grey) and soils incubated with leaf (green) or root (brown) litters during decomposition. Tables in each panel correspond to the effect of litter type (e.g. control, leaf or root litter) and time (e.g. 10, 38, 157 or 367 days) as revealed by two-way ANOVAs. Post-hoc tests investigating main effects of litter type or time are presented in Table 2. Different lowercase letters (panel a) indicate significant differences ($p < 0.05$, Tukey HSD) between each litter type × time combination. This post-hoc analysis was not performed for catabolic evenness as no significant effect of litter type × time was revealed by the two-way ANOVA.

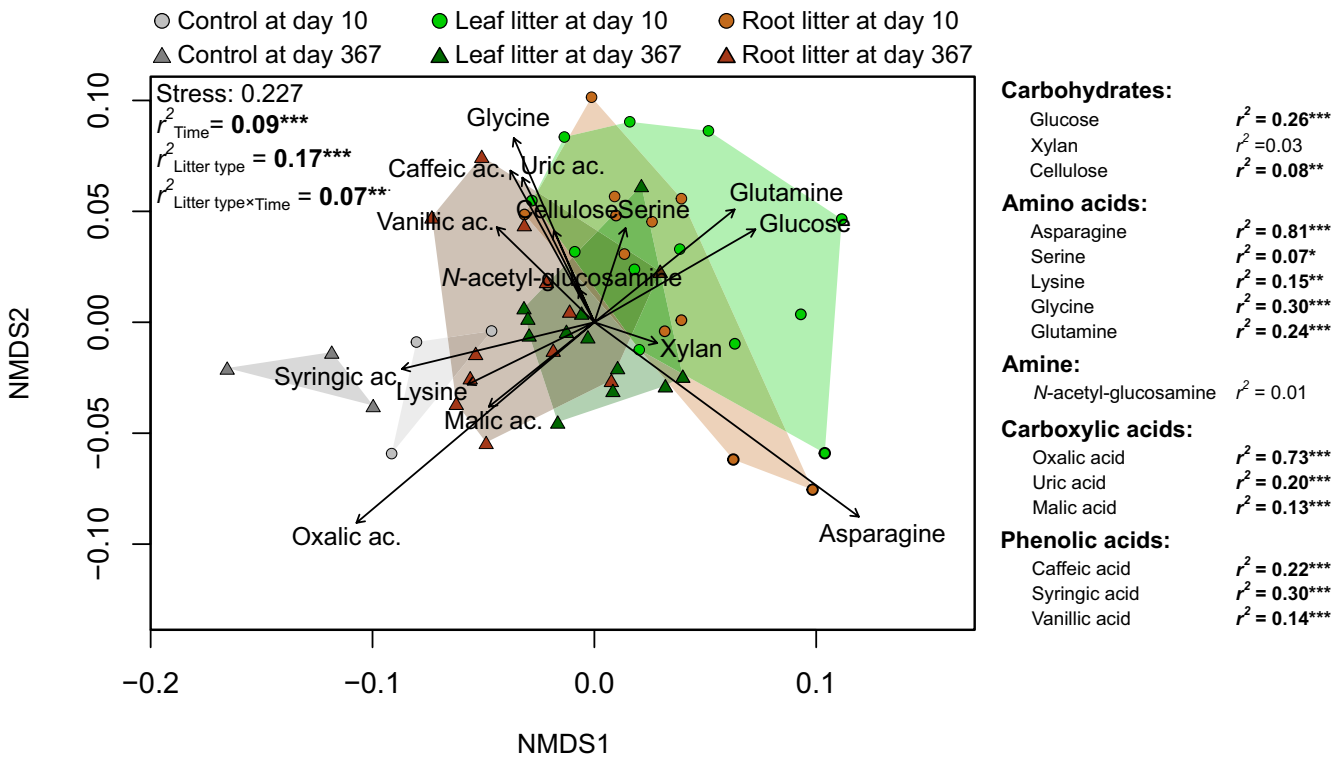

FIGURE 3 Catabolic capacities of soil microbial communities in soils incubated in different conditions (addition of leaf litter vs. root litter vs. pure soil) represented with a NMDS plot. Only the first (day 10, circles) and last (day 367, triangles) sampling times are visually represented here for clarity, but statistics were conducted on all sampling times. With increasing distance between two points, the catabolic capacities of microbial communities become more dissimilar. Vectors represent the goodness of fit statistics (r^2) of relative substrate-induced respiration of specific substrates fitted to the NMDS and the significance of their difference between all groups (time × litter type). PERMANOVA was conducted to test the significance of observed dissimilarities over differing sampling dates (r^2_{Time}), litter type ($r^2_{\text{Litter type}}$) and their interaction ($r^2_{\text{Litter type×Time}}$). * and *** show significance levels $p < 0.05$ and $p < 0.001$, respectively.

TABLE 3 Effect of litter type (root litter, leaf litter or control) at each sampling time (10, 38, 157 or 367 days) on catabolic profiles of microbial communities (based on their ability to degrade different substrates) as revealed by pairwise PERMANOVA performed on Bray–Curtis dissimilarity matrix. The relative contribution of each catabolic capacity (%) to the dissimilarity between each pairwise treatment (i.e. leaf, root and control) and for each sampling date (i.e. 10, 38, 157 and 367) were investigated using a SIMPER. A higher % means a higher contribution of a catabolic capacity to the difference between two treatments. †, *, ** and *** show significance levels at $p<0.1$, $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

	PERMANOVA				Glucose		Xylan		Cellulose		Asparagine		Serine		Lysine		Glycine	
	df	*F.model*	R^2	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*
Leaf vs. Root																		
10 days	1	**2.2**	**0.09**	*	1.0		0.7		0.5		1.6		0.6		0.5		0.5	
38 days	1	0.7	0.03		1.1		0.7		0.4		1.2		0.5		0.5		0.5	
157 days	1	**3.5**	**0.14**	**	1.2	†	0.7		0.5	†	1.6		0.8	†	0.4		0.6	
367 days	1	1.6	0.07		0.6		0.5		0.4		1.1		0.7		0.4		0.4	
Leaf vs. Control																		
10 days	1	4.8	**0.27**	**	1.0		0.6		0.5		1.7		0.5		**0.7**	*	0.6	
38 days	1	6.3	**0.33**	**	**1.5**	*	0.5		0.4		**2.4**	*	0.6		0.6		**0.9**	*
157 days	1	6.0	**0.32**	**	1.4	†	0.5		0.6	†	**3.4**	**	0.8		0.4		**1.0**	**
367 days	1	6.8	**0.35**	**	0.5		0.4		0.3		2.2	†	0.6		**0.8**	**	0.7	
Root vs. Control																		
10 days	1	4.2	**0.24**	**	0.7		0.5		0.6		2.1		0.5		0.5		0.5	
38 days	1	7.4	**0.36**	**	1.3		0.7		0.5		**2.8**	*	0.6		0.6		**1.0**	**
157 days	1	7.7	**0.37**	**	1.0		0.5		0.3		**3.0**	**	0.7		0.4		0.8	†
367 days	1	3.4	**0.21**	**	0.5		0.5		0.3		1.6		0.7		**0.8**	**	0.7	

	Glutamine		N-acetyl-glucosamine		Oxalic acid		Uric acid		Malic acid		Caffeic acid		Syringic acid		Vanillic acid	
	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*
Leaf vs. Root																
10 days	0.9		0.6		0.5		0.3		0.7		0.5		0.5		**0.8**	*
38 days	1.0		0.5		0.9		0.4		0.9		0.4		0.4		0.6	
157 days	1.2	†	0.6		0.8		0.4		0.9		0.5		0.5		0.5	
367 days	0.6		0.4		0.8		**0.6**	*	0.8		0.4		0.5		0.3	
Leaf vs. Control																
10 days	0.8		0.3		**2.6**	***	0.3		0.7		0.5		0.9	†	0.7	
38 days	**1.8**	**	0.7		**2.6**	***	0.6	†	**1.2**	*	0.4		**1.1**	*	0.5	
157 days	0.9		0.6		0.7		0.5		**1.3**	*	**1.4**	***	**1.1**	*	**1.2**	**

(Contiunes)

TABLE 3 (Contiuned)

	Glutamine		N-acetyl-glucosamine		Oxalic acid		Uric acid		Malic acid		Caffeic acid		Syringic acid		Vanillic acid	
	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*	Contribution (%)	*p*
367 days	1.3		0.3		**1.8**	*	0.5		0.8		0.7	†	**1.0**	*	0.5	
Root vs. Control																
10 days	1.2		0.5		**2.5**	***	0.3		0.5		0.6		0.8	†	0.5	
38 days	**1.8**	**	0.7		**2.7**	**	0.5		1.0		0.4		**1.1**	*	0.5	
157 days	**1.8**	**	0.6		1.0		0.5		**1.9**	***	**1.3**	***	**1.1**	*	**1.1**	*
367 days	1.0		0.4		1.6		0.4		0.8		0.6		0.8	†	0.5	

Significant values at $p < 0.5$ are highlighted in bold.

While the SIMPER procedure could identify one substrate driving this dissimilarity at day 10 (i.e. vanillic acid, Table 3), this was not the case for dissimilarity at day 157.

3.3 | Effect of time on catabolic capacities of microbial communities

Throughout the decomposition process, SIR_{tot} of soil mixed with either leaf or fine root litter decreased over time (Figure 2a; Table 2b). At 10 and 38 days, they were significantly higher than at all other sampling times. In contrast, time did not have a strong effect on catabolic evenness of the soil microbial communities. Only catabolic evenness of microbial communities at day 38 was significantly lower than that at day 367 (Figure 2b; Table 2b).

Catabolic profiles of microbial communities differed between sampling times (Figure 3; Table S6). For soil mixed with leaf litter, the dissimilarity between catabolic profiles along time was mainly related to differences in carbohydrates, amino acids and carboxylic acids degradation. More specifically, these dissimilarities were driven by catabolic capacities of microbial communities to degrade xylan (day 10 vs. 38 and day 10 vs. 367), cellulose (day 157 vs. 367), glycine (day 10 vs. 157), glutamine (day 38 vs. 157), malic acid (day 38 vs. 157) and oxalic acid (day 10 vs. 367, Table S6). Regarding soil mixed with root litter, the catabolic profile of microbial communities at day 367 was significantly dissimilar to all other sampling times. More specifically, differences in catabolic profiles were driven by catabolic capacities of microbial communities to degrade oxalic acid (10 vs. 367 days) and serine (day 157 vs. 367, Table S6). Additionally, catabolic profiles at day 10 and 157 were significantly dissimilar with one another, which was driven by differences in *N*-acetylglucosamine catabolic capacity (Table S6).

Overall, both the total catabolic activities and the catabolic profiles of microbial communities of soils mixed with litters showed a tendency to approach this recorded on pure soil at the start of the experiment (at day 10), but still differed substantially from these of pure soil at the end of the incubation (day 367) (Figures 2 and 3; Tables 2 and 3).

4 | DISCUSSION

Our main objective with this study was to assess how leaf and fine root litter chemical traits could alter catabolic capacities of soil microbial communities over the course of the decomposition process. A main finding of our experiment was that total catabolic activity at the early stages of decomposition was mainly influenced by the amount of C and N in litter leachates and litter N, P and Mg, and at later stages by litter lignin concentration. Catabolic capacities of microbial communities were also influenced by the concentration of minor elements in the litter material that are required for specific enzyme activities, such as Mn and Ca. Although litter traits were important drivers explaining catabolic capacities of the soil microbial

community from early to late decomposition stages, we found that litter type (i.e. leaf vs. root) had only minor effects on total catabolic activities or catabolic evenness. Furthermore, we observed that catabolic capacities in soils with ongoing litter decomposition progressively approached that measured in pure soil at the beginning of the experiment but still differed substantially. This dynamic response points at a tight connection of microbial metabolism to litter availability and quality. Overall, our results imply that besides its direct effect on the decomposition process, the chemistry of litter has a substantial indirect effect on litter decomposition via its influence on catabolic capacities of microbial communities. Most importantly, this effect faded with time, but remained substantial throughout the litter decomposition process and left legacy effects.

4.1 | Litter chemistry influence on catabolic capacities of microbial communities

In support of our first hypothesis, differences in litter chemistry underlying differences among species influenced both the total catabolic activity and specific catabolic capacities involved in the degradation of different C compounds (e.g. carbohydrates, amino acids, carboxylic acids and phenolic acids). Some of the differences observed when considering the full spectrum of leaf and root litter chemical traits and leaf and root litter considered separately were likely due to variable breadth of chemical differences across groups, and lower number of observations for the latter two. Overall, and most importantly, the influence of litter chemical traits changed over the course of the decomposition process. Copiotrophic communities that usually develop on litter at early decomposition stages are known to rely on high nutrient concentrations (e.g. N and P, Ramirez et al., 2012) and easily degradable C-sources to maintain high growth rates (Fanin et al., 2014). In partial support of this prediction, we observed a positive influence of litter N and N leachates on total catabolic activity at early stages of decomposition (days 10 and 38), likely because readily available N sources relieved soil microorganism growth limitation. However, litter P and litter C leachate concentrations showed a negative influence despite the importance of these elements for microbial activity (Fanin et al., 2016). As decomposition proceeded, lignin showed a positive influence on total catabolic activity at the intermediate decomposition stage (day 157), suggesting that microbial communities started to degrade lignin to get access to more recalcitrant C compounds once the pool of labile C compounds was partly depleted.

In addition to the predominant role of CNP in driving catabolic capacities, we also found that some micro-nutrients were important as well in explaining catabolic capacities of microbial communities. In particular, we observed a strong influence of litter Mn and Ca concentrations on specific catabolic capacities involved in the degradation of glucose and cellulose. Litter Ca content can increase the efficiency of cellulase to bind with cellulose, thus enhancing the abilities of microbial communities to depolymerize cellulose (Yousef et al., 2019). Regarding the positive effect of Mn, it is an element necessary for the synthesis of manganese peroxidase, a major enzyme involved in the degradation of lignin (Keiluweit et al., 2015; Thevenot et al., 2010). Thus, an increased Mn concentration might influence lignin degradation and lead to further cascading effects by releasing the protective effect of lignin on other C-sources (Fanin & Bertrand, 2016).

4.2 | Litter type influence on microbial communities

In contrast to our second hypothesis, litter type (i.e. root or leaf litter) had no clear influence on total catabolic activity or catabolic evenness. This is surprising given that the concentrations of C and N in litter leachates, lignin, N, Mn and Mg concentrations differed strongly between leaf and root litter (Table 1), as typically observed in previous studies (Freschet et al., 2012a; Sun et al., 2018). The absence of an effect may suggest that the differences in litter chemistry between leaves and roots are not large enough to generate a significant difference in the catabolic abilities of soil microbial communities, or that contrasting aspects of root versus leaf litter differences in chemistry have opposite influences on these activities. Furthermore, we mixed litter cut into small pieces to the soil, which may have artificially reduced the differences in substrate accessibility between leaves and roots. Altogether, these results suggest an important overlap between leaf and root chemical traits in influencing the catabolic capacities of microbial communities. Further differences between leaf and root litter may nonetheless appear in field conditions owing to differences in the localization of litter in the soil profile, an aspect that was deliberately standardized in this study to focus on chemical differences.

Although we did not find a significant effect on total catabolic activity, litter type influenced to some extent specific catabolic activities. After litter additions, the catabolic profile of microbial communities showed a rapid change compared with pure soil at day 10, suggesting a quick response of microbial communities to newly available substrates (Fanin et al., 2014). This effect was greater after leaf than after root additions, and the difference between leaves and roots was mainly driven by the catabolic capacities of microbial communities to degrade vanillic acid, the most recalcitrant lignin subunit (Thevenot et al., 2010). This effect of litter type on vanillic acid degradation is probably due to the release of oxidative enzymes for improved access to more accessible C compounds, which is likely faster for leaf than for root litter. Indeed, leaf litter tends to show a sharper decrease in chemical quality during decomposition (Amin et al., 2014; Sauvadet et al., 2019). On the other hand, differences between leaf and root litters at a later stage of decomposition were driven by their ability to degrade glucose, cellulose, serine and glutamine. This effect of litter type on structural C compounds and proteins may be due to the fact that leaves present greater resource availability and stimulated microbial activity over a longer period than roots (Sauvadet et al., 2019).

4.3 | Temporal dynamics on microbial community functions

In support of our third hypothesis, the influence of initial litter chemistry on microbial communities decreased over time. The decreased total catabolic activity and influence of litter chemistry with time are indicative of a reduced activity due to the exhaustion of litter-derived C-sources as litter was almost completely degraded after 367 days (i.e. 3% of litter C mass remaining at the end of the incubation period). At the start of the decomposition process, litter additions also strongly affected catabolic profiles of microbial communities compared with their 'original catabolic profile' (i.e. pure soil at day 10). These initial differences then got weaker with time, with catabolic profiles of microbial communities tending to approach their original profile towards the end of the experiment with only little litter left, especially for microbial communities incubated with roots. A similar trend has also been observed for the taxonomic composition of microbial communities that got taxonomically similar over time (Sauvadet et al., 2019), suggesting parallel shifts in microbial community composition and catabolic activities. Nonetheless, substantial differences in soil microbial community catabolic profiles were still present at the end of the litter decomposition process (especially when comparing soils mixed with litter with pure soils at the end of the incubation, which we took here as the baseline for identifying legacy effects of litter on soil microbial communities), suggesting a substantial legacy effect of litter on microbial catabolic activities. Such legacy effect has also been previously suggested for microbial community composition (Tardy et al., 2015).

Moreover, it is important to note that our experimental conditions accelerated decomposition considerably compared with what is typically observed in the field (Birouste et al., 2012; Kazakou et al., 2009; Pérez Harguindeguy et al., 2015; Table S7), suggesting that the temporal dynamics observed here may stretch over longer time periods under natural conditions. In natural ecosystems subjected to continuous or seasonal litter production, the litter pool would be typically replenishing at intervals that are shorter than the decreasing litter effects observed here. As such, the effects of litter on catabolic capacities of microbial communities observed here in a simplified experimental system are likely to appear even stronger and more persistent in the field. However, it remains unclear how litter inputs from ecosystems with diverse plant communities will interact to drive such legacy effects. In this context, one also needs to consider the large phenological variation in litter production between species (An et al., 2017) that can lead to staggered litter inputs to soil even in ecosystems dominated by seasonal litter production. Indeed, staggered litter inputs should broaden the diversity of niches available to microbial communities (Chapman et al., 2013; Chapman & Newman, 2010) by favouring the coexistence of litters at different stages of decomposition and distinct chemical composition, which may help maintain high levels of diversity in catabolic activities. Finally, as our study excluded environmental variations, which are known to influence the composition and activities of microbial communities, particularly in ecosystems experiencing seasonal climatic variations (Bardgett et al., 2005), it remains to be tested to what extent climatic variations would interact with the legacy effects of litter chemistry on soil microbial communities to dampen, cancel out, or may be even reinforce these effects in more realistic, in situ conditions.

AUTHOR CONTRIBUTIONS

Grégoire T. Freschet conceived the experiment, with input from Catherine Roumet, Stephan Hättenschwiler and Nathalie Fromin. Malo Y. Bourget, Grégoire T. Freschet and Catherine Roumet performed the experiment. Malo Y. Bourget and Ammar Shihan analysed the samples. Malo Y. Bourget, Nicolas Fanin, Raoul Huys and Grégoire T. Freschet analysed the data. Malo Y. Bourget wrote the first draft of the paper. All authors contributed to the writing of the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available in the Dryad Digital Repository https://doi.org/10.5061/dryad.0p2ngf25m (Freschet, 2023).

ORCID

Malo Y. Bourget https://orcid.org/0000-0001-8061-4536
Nicolas Fanin https://orcid.org/0000-0003-4195-855X
Catherine Roumet https://orcid.org/0000-0003-1320-9770
Raoul Huys https://orcid.org/0000-0002-7499-674X
Marie Sauvadet https://orcid.org/0000-0002-7520-8565
Grégoire T. Freschet https://orcid.org/0000-0002-8830-3860

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1: Correlation between initial litter chemical traits.

Table S2: Effects of species identity, sampling time (10, 38, 157 or 367 days) and litter type (root or leaf), on total catabolic activities (SIR_{tot}) and catabolic evenness (H').

Table S3: Effect of species identity at each sampling time (10, 38, 157 or 367 days) on catabolic profiles of microbial communities as revealed by pairwise PERMANOVA performed on Bray–Curtis dissimilarity matrix.

Table S4: Effects of plant functional type (legume, forb or graminoid), sampling time (10, 38, 157 or 367 days) and litter type (root or leaf), on total catabolic activities (SIR_{tot}) and catabolic evenness (H').

Table S5: Effect of plant functional type (legume, forb or graminoid) at each sampling time (10, 38, 157 or 367 days) on catabolic profiles of microbial communities as revealed by pairwise PERMANOVA performed on Bray–Curtis dissimilarity matrix.

Table S6: Effect of time (10, 38, 157 or 367 days) for each litter type (root or leaf litter) on catabolic profiles of microbial communities (based on their ability to degrade different substrates) as revealed by pairwise PERMANOVA performed on Bray–Curtis dissimilarity matrix.

Table S7: Decomposition rates *k* and half-lives of leaf and root litters from the current study and previous studies.

Figure S1: Cumulative decomposed litter C for leaf (left column) and root (right column) litter for all 12 species expressed as a fraction of the initial litter C.

Figure S2: Heatmaps of the estimated effects of litter initial traits on microbial community relative substrate induced respiration (rSIR) at day (A) 10, (B) 38, (C) 157 and (D) 367.

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