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### Official URL:

<https://doi.org/10.1007/s00248-019-01353-3>

### To cite this version:

Jabiol, Jérémy and Lecerf, Antoine and Lamothe, Sylvain and Gessner, Mark O. and Chauvet, Eric Litter Quality Modulates Effects of Dissolved Nitrogen on Leaf Decomposition by Stream Microbial Communities. (2019) *Microbial Ecology*, 77 (4). 959-966. ISSN 0095-3628

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# Litter Quality Modulates Effects of Dissolved Nitrogen on Leaf Decomposition by Stream Microbial Communities

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## Abstract

Rates of leaf litter decomposition in streams are strongly influenced both by inorganic nutrients dissolved in stream water and by litter traits such as lignin, nitrogen (N) and phosphorus (P) concentrations. As a result, decomposition rates of different leaf species can show contrasting responses to stream nutrient enrichment resulting from human activities. It is unclear, however, whether the root cause of such discrepancies in field observations is the interspecific variation in either litter nutrient or litter lignin concentrations. To address this question, we conducted a controlled laboratory experiment with a known fungal community to determine decomposition rates of 38 leaf species exhibiting contrasting litter traits (N, P and lignin concentrations), which were exposed to 8 levels of dissolved N concentrations representative of field conditions across European streams (0.07 to 8.96 mg N L<sup>-1</sup>). The effect of N enrichment on decomposition rate was modelled using Monod kinetics to quantify N effects across litter species. Lignin concentration was the most important litter trait determining decomposition rates and their response to N enrichment. In particular, increasing dissolved N supply from 0.1 to 3.0 mg N L<sup>-1</sup> accelerated the decomposition of lignin-poor litter (e.g. < 10% of lignin, 2.9× increase ± 1.4 SD, *n* = 14) more strongly than that of litter rich in lignin (e.g. > 15% of lignin, 1.4× increase ± 0.2 SD, *n* = 9). Litter nutrient concentrations were less important, with a slight positive effect of P on decomposition rates and no effect of litter N. These results indicate that shifts in riparian vegetation towards species characterized by high litter lignin concentrations could alleviate the stimulation of C turnover by stream nutrient enrichment.

**Keywords** Litter breakdown · Nutrient enrichment · Freshwater fungi · Litter lignin · Michaelis–Menten–Monod kinetics · Litter traits

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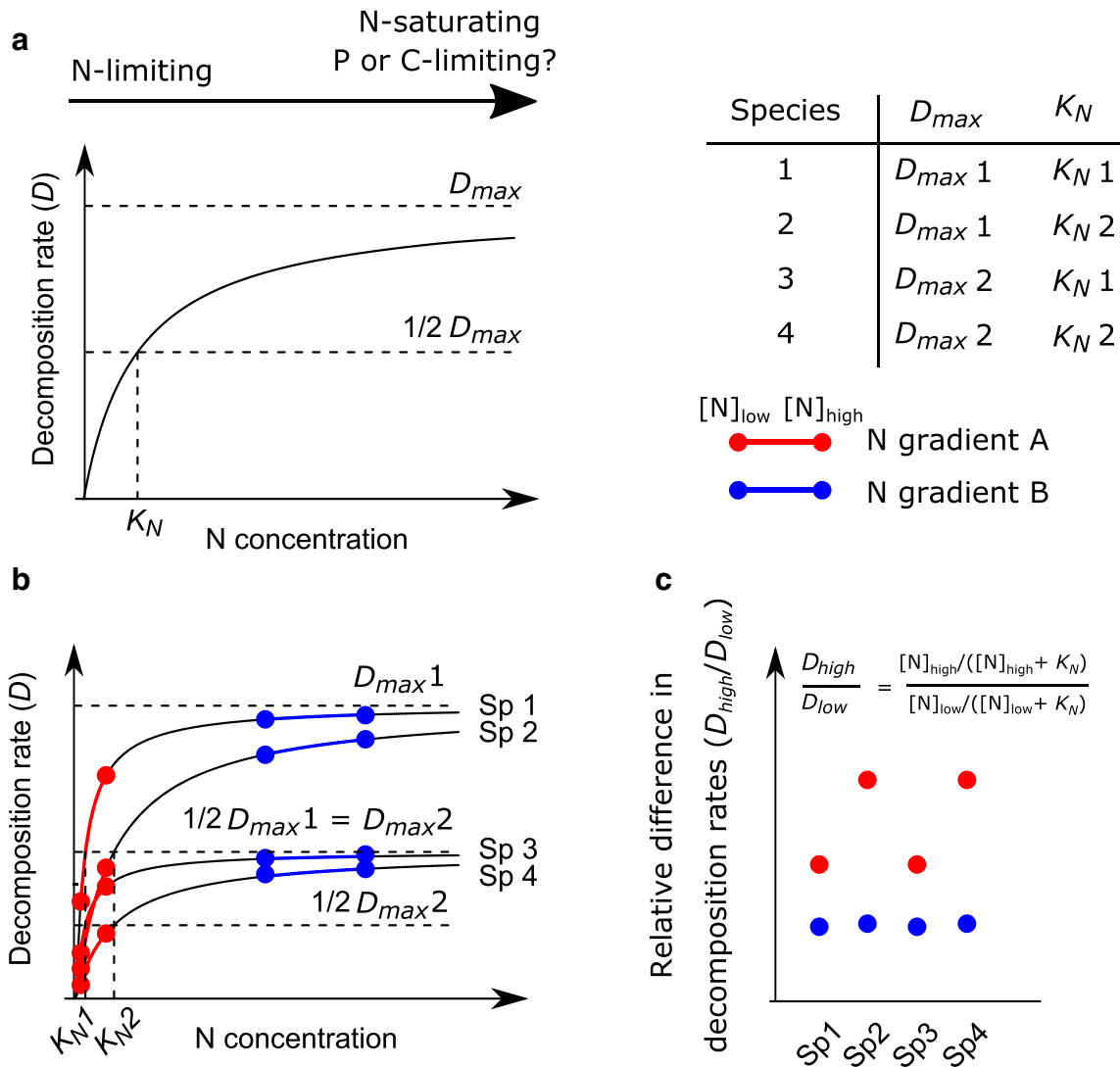
## Introduction

Plant litter decomposition is a key process in the global carbon (C) cycle [1] that fuels the food webs of woodland ecosystems, including their headwater streams [2–5]. Typically depleted in nutrients relative to C, leaf litter imposes strong nutrient constraints on microbial decomposers [6, 7] and detritivores [8–10]. Among the potentially limiting nutrients, nitrogen (N) and phosphorus (P) play particularly important roles in supporting biological activities. These elements promote the growth of organisms (particularly P, as encapsulated in the growth rate hypothesis [11], but also N), since both are major constituents of nucleic acids (9.2% P, 15.7% N), phospholipids (4–5% P) [12] or proteins (17.2% N in amino acids) and tend to be scarce in ecosystems.

As a result of nutrient limitation, plant litter with high nutrient concentrations tends to decompose faster

than nutrient-depleted litter [13]. In streams and rivers, however, water flow continuously replenishes the dissolved nutrient supply available to microbial decomposers, which immobilize these nutrients [14, 15] and are thus able to cope with the strong nutrient imbalances in plant litter. Aquatic hyphomycetes—the dominant microbial decomposers of leaf litter in streams—can indeed strongly rely on dissolved nutrients [16, 17]. This explains the stimulation of microbial activity on leaf litter by external nutrient supply [18], resulting in higher fungal biomass [19], reproduction [14] and rates of leaf litter decomposition [20, 21].

The effects of external nutrients on litter decomposition in streams are well described by the Monod equation, which adequately models decomposition rate as a function of nutrient supply (e.g. [18, 20]). Accordingly, decomposition rate asymptotically approaches a maximum,  $D_{max}$ , as N supply increases (Fig. 1). The theoretical underpinning is that, all else being equal, microbial activity and thus decomposition rate shift from N limitation to limitation by other factors as N supply gradually approaches satiating levels. The half-saturation constant ( $K_N$ ) is the N concentration where decomposition rate is half of its maximum,  $D_{max}$  (Fig. 1a).  $K_N$  can also be viewed as embodying the relative increase in litter



**Fig. 1** **a** Monod equation showing decomposition rate as a function of dissolved N supply, from N-limiting to N-saturating concentrations. The figure displays  $D_{max}$  (decomposition rate at the asymptote) and the half-saturation constant,  $K_N$ , which is the dissolved N concentration at which decomposition rate equals  $1/2 D_{max}$ . **b** Monod models for four hypothetical litter species with different combinations of  $D_{max}$  and  $K_N$ , with  $D_{max} 1 = 1/2 D_{max} 2$ . Red and blue scenarios correspond to N supply gradients used to illustrate the sensitivity of decomposition rate of the four

species to N enrichment. **c** The fact that—according to the Monod model—strength of the N effect on the decomposition of a given litter species, which is calculated as the ratio of decomposition rates at high and low N availability ( $D_{high}$  and  $D_{low}$ , respectively), only depends on the magnitude of N enrichment (blue vs. red scenario) and the half-saturation constant  $K_N$ , but is independent of  $D_{max}$  (Sp1 = Sp3 ≠ Sp2 = Sp4)

decomposition rate along the N concentration gradient, i.e. the sensitivity of decomposition rate to N supply. Figure 1b, c illustrates that, according to the Monod equation, the dissolved N effect on decomposition rate depends on the N enrichment level (stronger when the basal nutrient concentration is low), and on  $K_N$ , with stronger N effects for litter species with high  $K_N$  (i.e. Sp 2 and 4). Counterintuitively, the relative increase in decomposition rate along a given N enrichment gradient is not affected by  $D_{max}$ , resulting in the same N effect for both the species pair 1 and 3 and the species pair 2 and 4 in Fig. 1.

Another key driver of leaf decomposition is the quality of C compounds in leaf litter, which is inversely related to concentrations of structural leaf constituents such as cellulose, hemicellulose and lignin. Lignin, in particular, typically accounts for 5–35% of leaf litter dry mass and is a key predictor of litter-associated microbial activity and decomposition rates (e.g. [22–24]). Lignin is a heterogeneous polymer that is highly resistant to enzymatic degradation involving a complex set of enzymes synthesized by fungi and bacteria (peroxidases, laccases and other phenoloxidases [25]). Moreover, because of the intimate association of lignin with cellulose and hemicellulose, high lignin concentrations also limit the accessibility of those more readily available C sources to decomposers [26].

Effects of external nutrient supplies [17, 21, 27] and litter lignin and nutrient concentrations [9, 23] on decomposition have been previously reported. However, the relative importance of external and internal nutrient pools and the extent to which litter lignin concentration modulates nutrient effects on decomposition in streams remain unknown [27]. Where nutrient enrichment effects on multiple litter species have been investigated, they were mostly found to be greater for low-quality litter (e.g. [18, 20, 28]), although the opposite pattern has also been noted [29]. All previous studies, however, involved at most four leaf species. This strongly narrows the scope of inferences that can be drawn and also prevented disentangling the roles of individual litter traits such as lignin, N and P concentrations in determining responses to nutrient enrichment.

These limitations can be overcome by examining nutrient effects over broad gradients of nutrient concentrations and litter qualities of multiple leaf species where individual traits are uncorrelated. Thus, the objective of the present study was to unravel the importance of chemical litter quality defined by N, P and lignin concentrations on the effects of external N supply on litter decomposition. Using the Monod model to describe the relationship between dissolved N concentration and decomposition rate, we examined variation in the parameters  $D_{max}$  and  $K_N$  (Fig. 1) across 38 litter species exhibiting contrasting lignin and nutrient (N and P) concentrations. We expected lignin to slow decomposition (i.e. low  $D_{max}$  of litter rich in lignin) by hampering C utilization to decomposers and,

thus, also to decrease N demand in support of microbial growth. As a consequence, the effect of N on decomposition rates should be weaker on lignin-rich litter (i.e. low  $K_N$  of litter rich in lignin). Since litter N can compensate for a short supply of external N, we also expected that litter N would positively influence litter decomposition at low but not high dissolved N concentrations. Dissolved N effects on decomposition rate should also be weaker for N-rich litter, resulting in a lower  $K_N$ . Finally, P may become limiting to microbial decomposers when external N is provided in excess. As a consequence, high litter P concentrations should have a positive effect on decomposition rate at high dissolved N concentrations (i.e. positive effect on  $D_{max}$ , especially on lignin-poor litter), and the dissolved N effects should be stronger when P is not limiting (i.e. on P-rich litter), translating into a positive effect of litter P on  $K_N$ .

## Material and Methods

Senescent leaves of 38 common woody plant species (Supplementary Material 1) were collected in autumn 2017 at multiple locations in south-western France. Senescent leaves were picked from the plants just before abscission when a parting tissue had formed, or they were collected from the ground just after abscission. The leaves were air-dried in the laboratory, and representative aliquots of each species were ground with a mixer mill (Retsch MM200, Haan, Germany). The ground material was used to determine litter chemistry. Proximate lignin concentration was determined following the acid-detergent fibre procedure by Van Soest [30]; litter N concentration was measured with a CHN analyser (Flash 200, Thermo Fisher Scientific, Waltham, MA, USA) and P litter concentration spectrophotometrically (Uvi Light XT5, Secomam, Alès, France) after digestion with persulfate. The litter trait values are presented in Supplementary Material 1.

Thirty-six batches of two leaf discs of each species were cut with a cork-borer (10 mm diameter) and weighed to the nearest 0.01 mg after 48 h of drying at 40 °C. The batches were randomly allocated to wells of 12-well microplates (21 mm diameter) filled with 2 mL of demineralized water, and incubated on an orbital shaker (75 rpm) at ca. 14 °C (mean  $\pm$  SD = 14.4  $\pm$  0.5 °C). After 48 h, four replicate batches of two discs each of all litter species were randomly chosen, dried at 40 °C for 48 h and weighed to the nearest 0.01 mg to determine mass loss due to leaching.

The water in the remaining wells was sucked off with a vacuum pump and replaced by 2 mL of a fungal spore suspension. The spore suspension was obtained from naturally decomposing leaves collected in the Peyreblanque stream in south-western France (43° 25' 41" N, 2° 13' 13" E, 738 m a.s.l.). Peyreblanque is a forested headwater stream bordered by a diverse riparian community (~10

tree and shrub species dominated by *Fagus sylvatica*, *Corylus avellana*, *Salix caprea* and *Quercus robur*) and exhibiting intermediate dissolved N concentrations compared to our experimental N gradient (0.75 mg L<sup>-1</sup> on average, based on 15 analyses carried out year-round before the beginning of the experiment). Decomposing leaves collected in the stream were carried to the laboratory and submerged for 3 days in 3 L of demineralized water with constant shaking and aeration at a temperature of ca. 14 °C. The spore density and species composition of aquatic hyphomycetes in the suspension were determined just before inoculation of the microplate wells by filtering ten replicate 2-mL aliquots of the suspension over a nitrocellulose membrane (5 µm pore size; Whatman International Ltd., Maidstone, UK). The spores were stained with Trypan blue (0.5% in 60% lactic acid) and counted and identified at ×200 magnification. The 2-mL aliquots contained 11,473 ± 1337 (SD) spores belonging to an average of 18.2 ± 1.7 (SD) species of aquatic hyphomycetes (Supplementary Material 2). The identified species are among the most common in temperate forest streams [31], reflecting particularly well the composition of aquatic hyphomycete communities in streams of the Montagne Noire (south-western France) [32]. After 48 h, the spore suspensions were replaced by mineral solutions with variable N concentrations.

Mineral solutions were adapted from Suberkropp et al. [33]. They contained 174 µg K<sub>2</sub>HPO<sub>4</sub>, 7.4 mg CaCl<sub>2</sub>, 7.4 mg MgSO<sub>4</sub>·7H<sub>2</sub>O, 8.5 mg NaHCO<sub>3</sub>, 0.2 mg MnSO<sub>4</sub>·H<sub>2</sub>O, 0.2 mg ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.02 mg Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.02 mg KI, 0.008 mg CoCl<sub>2</sub>·6H<sub>2</sub>O and 0.005 mg NiCl<sub>2</sub>·6H<sub>2</sub>O in 1 L of demineralized H<sub>2</sub>O. KNO<sub>3</sub> was supplied at eight different levels corresponding to final N concentrations of 0.07, 0.14, 0.28, 0.56, 1.12, 2.24, 4.48 and 8.96 mg L<sup>-1</sup>. Mineral solutions were autoclaved (121 °C, 20 min) before being distributed to the wells. Four replicates per litter species received 2 mL of each of the eight mineral solutions (i.e. 1248 samples in total), which were renewed twice a week throughout the experiment.

Decomposition of the different litter species was stopped sequentially when ca. 30% of post-leaching leaf mass loss had occurred in the mineral solution with the highest N concentration, as determined from one extra batch of leaf discs per species. After 67 days of decomposition, the experiment was stopped also for the seven remaining litter species that had not reached this decomposition stage. The leaf discs were dried at 40 °C for 48 h and weighed to the nearest 0.01 mg. Leaf decomposition rate (i.e.  $D$ ) was expressed as the leaf dry mass lost per day relative to the initial litter mass after leaching, assuming a linear decrease of litter mass over time. This was determined as the best estimate of leaf decomposition dynamics in our microcosm study as found in a preliminary

experiment carried out in the same conditions but with two litter species, two mineral solutions and five sampling dates (Supplementary Material 3). The mass loss of six samples (out of 1248) where leaf mass was higher at the end than at the beginning of the experiment was considered 0 in all analyses.

To assess the combined effect of litter quality and dissolved N concentration on litter decomposition of each litter species, we performed separate non-linear regression analyses to fit the data to a Monod-type model (Fig. 1),

$$D = D_{\max} \times \frac{[N]}{[N] + K_N},$$

where  $D$  is the decomposition rate (% day<sup>-1</sup>),  $D_{\max}$  is the decomposition rate when N is not limiting,  $[N]$  is the dissolved N concentration (mg L<sup>-1</sup>) and  $K_N$  is the half-saturation constant (mg N L<sup>-1</sup>), i.e. the N concentration at which  $D = 1/2 D_{\max}$  (Fig. 1).

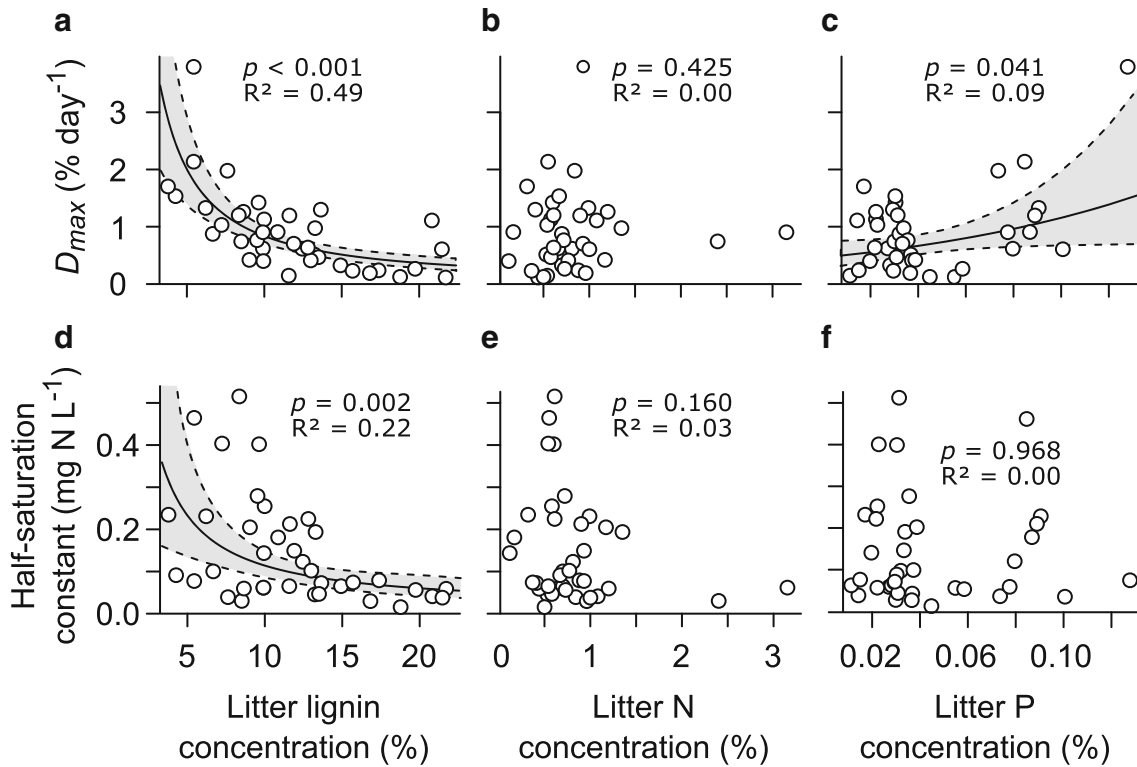
Variation in these parameters was then compared along the gradient of litter quality (lignin, N and P concentrations) using linear regressions after natural log transformation of  $D_{\max}$ ,  $K_N$  and litter lignin concentration. All analyses were performed using R 3.3.0 [34].

## Results

The use of a large set of litter species enabled us to create broad gradients of three fundamental litter traits: lignin, N and P concentrations. Lignin concentration varied more than fivefold, from 4.0 to 21.9% of litter dry mass, and the ranges for N (0.15–3.18%) and P (0.012–0.129%) were even larger (Supplementary Material 1). Importantly, these three traits varied independently from one another ( $p > 0.28$ ), with Pearson correlation coefficients  $R < 0.18$  (Supplementary Material 1).

The decomposition experiment ran for 10 to 67 days (mean ± SD 40 ± 18 days), depending on litter species, which corresponds to a remaining litter mass in the high-N solution of 46–92% of the initial mass after leaching (mean ± SD 70 ± 10%). In the N-rich solution, mean decomposition rates ranged from 0.172% (*Quercus petraea*) to 4.26% (*Ulmus glabra*) of leaf mass loss per day.

The parameter estimates of the Monod equation ( $D_{\max}$  and half-saturation constant  $K_N$ ) were all significant except the  $K_N$  estimate for four species (Supplementary Material 1). Both parameters varied widely among litter species, ranging from 0.14 to 3.83% day<sup>-1</sup> and from 0.007 to 0.506 mg N L<sup>-1</sup>, respectively. As predicted, both  $D_{\max}$  and  $K_N$  were negatively related to litter lignin concentration (Fig. 2a, d), meaning that the decomposition rate of lignin-rich litter saturates at a lower decomposition rate and a lower dissolved N concentration



**Fig. 2** Variation in  $D_{max}$  and  $K_N$  of the Monod model along gradients of litter lignin, N and P concentrations. **a–c** The effect of litter quality on the maximal rate of litter decomposition ( $D_{max}$ ). **d–f** The effects of litter quality on the half-saturation constant ( $K_N$ ). Regression lines between

these parameters and litter lignin, N, or P concentrations are displayed when significant, with 95% CI shown as grey surfaces, together with the corresponding  $p$  and  $R^2$  values

than for lignin-poor litter species. Litter P concentration had a positive effect on  $D_{max}$  (Fig. 2c) but no significant influence on  $K_N$  (Fig. 2f). Finally, litter N concentration had no significant influence on either parameter of the Monod equation (Fig. 2b, e), suggesting that the litter N effect was negligible compared to the effect of dissolved N supply. The  $R^2$  and  $p$  values for these regressions were not altered when the non-significant estimates of  $K_N$  were excluded.

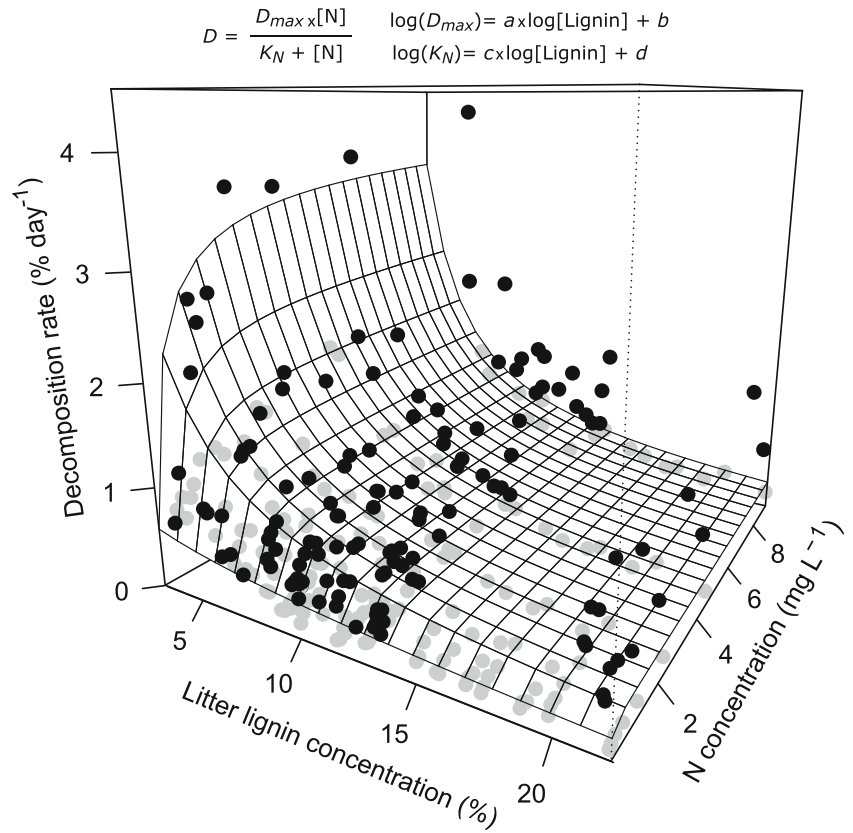
## Discussion

With 38 litter species spanning a wide gradient of litter qualities, this study provides robust evidence for the importance of litter quality in determining responses of decomposition to external N supply in streams (Fig. 3) over a realistic dissolved N gradient [35]. Our findings strongly support the hypothesis that the stimulatory effect of external N supply is weaker on lignin-rich than on lignin-poor leaf litter, as indicated by a negative relationship between litter lignin concentration and both parameters of the Monod equation, the maximum decomposition rate ( $D_{max}$ ) and the half-saturation constant ( $K_N$ ) (see Fig. 1). This pattern is likely to reflect a limitation of microbial decomposer activity by C availability: stimulated by N enrichment at low supply levels, decomposition quickly

shifts from N-limitation to C-limitation governed by lignin that not only is refractory but also restricts access to other more labile C sources, including cellulose and hemicellulose [26]. This C limitation by lignin thus drives the low half-saturation constant ( $K_N$ ), slow microbial growth and consequently low maximal litter decomposition rates ( $D_{max}$ ).

As indicated by relatively high half-saturation constants we found for some litter species (up to 0.51 mg N L<sup>-1</sup>) compared with previous estimates (0.016–0.026 mg N L<sup>-1</sup> [18]), the stimulating effect of dissolved N on litter decomposition persisted in some litter species even at high dissolved N concentrations. This outcome reinforces evidence that inorganic dissolved N in stream water [14, 15] is of prime importance for aquatic hyphomycetes decomposing submerged leaf litter [17, 36]. The high N demand by fungal decomposers we observed may exceed that expected from the Redfield atomic N:P ratio of 16:1, which in our experiment was reached between 0.2 and 0.4 mg N L<sup>-1</sup>. This is likely to reflect, in part, a high investment of N in the synthesis of extracellular enzymes needed to degrade leaf material. In addition, it could point to a notable stoichiometric flexibility of aquatic hyphomycetes with respect to P concentrations in their biomass [17, 37], which can be adjusted to molar C:P ratios of nearly 500 [17] or even 1500 [37] when P is limiting. Similar abilities to adjust their elemental composition have been reported for

**Fig. 3** Decomposition rate of litter as a function of dissolved N and litter lignin concentration. Each symbol denotes the average of four replicate microcosms. The three-dimensional surface shows the Monod model depicting the effect of litter lignin concentration on  $D_{max}$  and  $K_N$  following the formulas provided at the top of the figure. Parameters  $a$ ,  $b$ ,  $c$  and  $d$  are derived from the regression analyses described in Fig. 2a, d. Data points above and below the response surface are displayed in black and grey, respectively



communities of heterotrophic freshwater bacteria [38], suggesting that stoichiometric flexibility particularly with respect to P is a general feature of microbial decomposers in freshwaters, allowing them to cope with often very low and variable P availability in their environment.

Litter nutrients had a weaker influence on the dissolved N effects on decomposition than lignin. Contrary to our prediction, litter N availability did not shift the half-saturation constant ( $K_N$ ) over our litter N concentration range, indicating that litter N could not relax the limitation of decomposition rates imposed by low external N supply. In fact, except during early colonization stages, aquatic hyphomycetes rely little on litter nutrient pools compared to dissolved nutrients [16]. Both nutrient sources are exploited by means of distinct mechanisms involving different sets of enzymes and are likely to incur different energetic costs [17, 39]. Contrary to N, a positive effect of P was observed on  $D_{max}$ . This suggests that microbial communities were able to take advantage of the P released during leaf litter degradation, thus coping with the relative P deficiency that emerges as supply levels of dissolved N increase (i.e. at high N:P). However, since considerable amounts of litter P can be rapidly lost during initial leaching [40], the relationship we observed between decomposition rate and litter P concentration before leaching could be masked.

Past studies comparing the decomposition of a limited number of litter species exhibiting contrasting N

concentrations (e.g. alder vs. oak [18, 20]) concluded that stronger effects of dissolved N on the decomposition of refractory species indicate a relaxation of N limitation by high litter N concentrations [27]. Our results on decomposition rates of 38 litter species differing greatly in N concentrations (0.15–3.18% of pre-leaching litter dry mass; Supplementary Material 1) do not support the notion that this pattern holds generally. However, we found half-saturation constants remarkably close to values reported from a field experiment involving N enrichment of a stream [18] for litter of both alder (0.021 mg N L<sup>-1</sup> in our study vs. 0.016 mg N L<sup>-1</sup>) and oak (0.021 in our study vs. 0.026 mg N L<sup>-1</sup>). Although the sensitivity of litter decomposition to nutrient enrichment was unaffected by litter N concentration, decomposition of some litter species with particularly high N concentrations (e.g. N-fixers such as alder) could be less affected by N enrichment than most other litter species.

Our results appear to be in contrast to a strong stimulatory effect of dissolved nutrients on the decomposition of wood compared to leaves [18, 41, 42]. In particular, Ferreira et al. [18] found a higher half-saturation constant ( $K_N$ ) for balsa wood veneers than for oak leaves, although  $D_{max}$  was similar. This means that decomposition rates of wood saturate at a higher dissolved nutrient supply level than those of leaves, indicating that wood decomposition requires more external N to reach its maximum rate. This result is likely to reflect

the very low N concentration of wood [42] combined with a high N demand for the production of extracellular microbial enzymes to degrade wood polymers, particularly cellulose and lignin. Moreover, wood has a low surface-to-volume ratio, which in freshwater, where decomposition is restricted to a thin wood surface layer, limits microbial access to the bulk wood biomass [43].

Overall, our results are an important step towards unveiling the complex interplay between litter lignin concentration and nutrient availability (in both leaf litter and the environment) as drivers of litter decomposition by stream microbial communities. The quantitative predictions from our microcosm experiment have broad implications, since widespread nutrient mobilization and release by diverse human activities at local and global scales inevitably cause extensive nutrient enrichment of surface waters. Prominent effects on ecosystem processes include those on litter decomposition (e.g. [21, 35]). For example, our parameter estimates of the Monod model lead to the prediction that an increase in stream water concentrations from 0.1 to 3 mg N L<sup>-1</sup> [20] accelerates the microbial decomposition even of lignin-rich litter, such as beech and oak, by 1.5× and 1.2×, respectively, and that of some lignin-poor litter types such as cherry (*Prunus avium*) and elm (*Ulmus minor*) by as much as 4.5×. These increases match data from field investigations: an observational study using a moderate gradient of dissolved N and P concentrations in streams found a 1.3× and 1.8× stimulation of microbial decomposition of alder and oak litter, respectively [20], resembling responses of microbial decomposition (up to 1.3× higher respiration associated with decomposing litter) to experimental stream nutrient enrichment along a gradient from 88 to 517 μg N L<sup>-1</sup> [44].

A response to N enrichment to be expected at large scale is a global C loss from stream ecosystems [21], unless C retention is simultaneously enhanced through the stimulation of litter consumption by macro-consumers [20]. As our data suggest, the magnitude of this C loss appears to depend on the quality of the litter delivered by riparian vegetation. Litter quality varies substantially both between [3, 45] and within [24, 46, 47] species distributed over broad climatic gradients [24, 45], with higher lignin concentrations generally found in tropical and Mediterranean plants [48]. With global warming proceeding, northern shifts in the distribution of riparian tree and shrub species [49] and increased C allocation to structural leaf constituents in response to elevated atmospheric CO<sub>2</sub> concentrations [50] could increase the allocation of C to lignin at the expense of litter C compounds more readily available to decomposers. Under this scenario, higher litter recalcitrance could alleviate the stimulation of C turnover in response to increasing nutrient availability in stream ecosystems [44].

**Acknowledgments** The authors are grateful to Frédéric Julien and Wendy Ambilas for litter CNP analyses.

**Funding information** This study is part of the FunctionalStreams project funded by the French National Research Agency (grant ANR-14-CE01-0009-01).

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# Online Supplementary Material

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## **Litter quality modulates effects of dissolved nitrogen on leaf decomposition by stream microbial communities**

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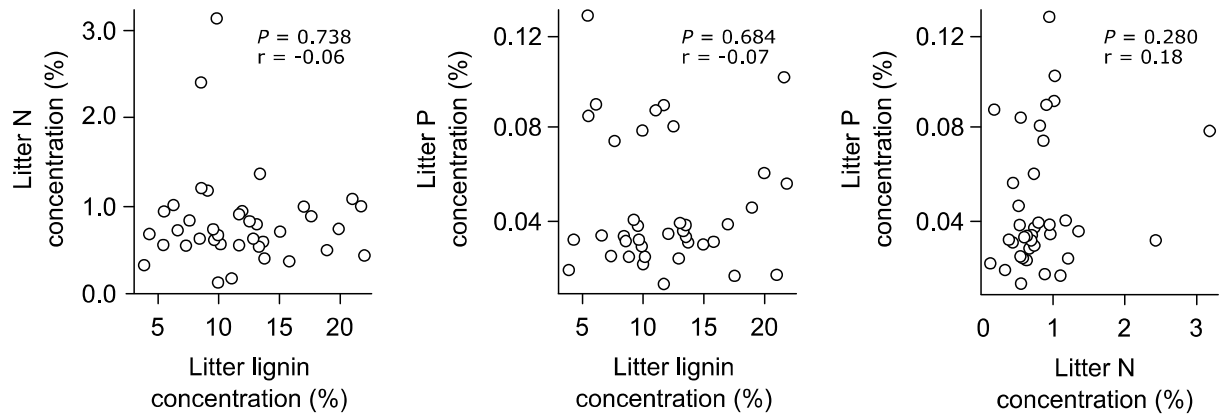
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## Supplementary Material 1

**Table S1** Duration of the experiment (days), chemical characteristics (% dry mass) and the estimated Monod parameters  $D_{max}$  (% day<sup>-1</sup>) and  $K_N$  (mg N L<sup>-1</sup>) that describe the effects of dissolved N on decomposition rate. Stars indicate significant estimates of  $K_N$  at  $\alpha = 0.05$  (estimates of  $D_{max}$  are all significant)

Family	Species	Duration	N	C	P	Lignin	$D_{max}$	$K_N$
Aceraceae	<i>Acer campestre</i> L.	55	0.84	45.78	0.08	12.62	0.65	0.114*
	<i>Acer monspessulanum</i> L.	27	0.61	45.31	0.02	10.16	1.16	0.246*
	<i>Acer platanoides</i> L.	24	0.63	43.56	0.03	9.80	1.45	0.393*
	<i>Acer pseudoplatanus</i> L.	39	0.69	47.27	0.03	10.08	0.65	0.052*
Anacardiaceae	<i>Pistacia terebinthus</i> L.	61	0.57	50.61	0.01	11.75	0.18	0.056*
Aquifoliaceae	<i>Ilex aquifolium</i> L.	39	0.61	48.41	0.03	13.67	0.50	0.038
Araliaceae	<i>Hedera helix</i> L.	24	0.44	47.36	0.03	13.82	1.33	0.064*
Betulaceae	<i>Alnus glutinosa</i> (L.) Gaertn.	33	2.43	47.29	0.03	8.68	0.77	0.021
	<i>Betula pendula</i> Roth.	67	0.73	47.80	0.03	15.09	0.36	0.056*
	<i>Carpinus betulus</i> L.	39	0.73	45.27	0.03	6.84	0.91	0.091*
	<i>Corylus avellana</i> L.	33	1.38	44.07	0.04	13.45	1.01	0.185*
Buxaceae	<i>Buxus sempervirens</i> L.	25	1.11	48.94	0.02	20.99	1.14	0.032*
Cornaceae	<i>Cornus sanguinea</i> L.	21	0.70	44.88	0.03	4.44	1.57	0.082*
Ericaceae	<i>Arbutus unedo</i> L.	55	0.15	46.59	0.02	10.11	0.43	0.135*
	<i>Vaccinium myrtillus</i> L.	33	3.18	42.99	0.08	10.08	0.94	0.053*
Fagaceae	<i>Castanea sativa</i> Mill.	46	0.56	48.62	0.04	13.40	0.55	0.037*
	<i>Fagus sylvatica</i> L.	67	0.47	48.57	0.06	21.88	0.14	0.050
	<i>Quercus ilex</i> L.	67	0.91	47.86	0.02	17.55	0.27	0.070*
	<i>Quercus petraea</i> (Matt.) Liebl.	67	0.53	49.45	0.05	18.94	0.15	0.007
	<i>Quercus pubescens</i> Willd.	67	0.40	47.95	0.03	15.87	0.26	0.065*
	<i>Quercus robur</i> L.	67	0.99	47.48	0.04	16.99	0.22	0.021*
Juglandaceae	<i>Juglans regia</i> L.	61	0.80	47.58	0.04	13.17	0.44	0.093*
Oleaceae	<i>Fraxinus excelsior</i> L.	27	1.23	41.42	0.02	8.82	1.29	0.051*
Rhamnaceae	<i>Frangula alnus</i> Mill.	17	0.87	44.86	0.07	7.79	2.01	0.030*
	<i>Rhamnus alaternus</i> L.	25	0.35	42.38	0.02	3.95	1.74	0.226*
Rosaceae	<i>Crataegus monogyna</i> Jacq.	55	0.75	44.37	0.04	9.70	0.80	0.270*
	<i>Prunus avium</i> (L.) L.	27	0.64	46.29	0.03	8.52	1.23	0.506*
	<i>Rubus ulmifolius</i> Schott	25	1.02	44.93	0.09	6.38	1.36	0.222*
	<i>Sorbus aria</i> (L.) Crantz	67	0.76	36.81	0.06	19.91	0.30	0.047*
	<i>Sorbus aucuparia</i> L.	39	1.03	35.39	0.10	21.64	0.64	0.029*
	<i>Sorbus torminalis</i> (L.) Crantz	61	1.20	44.27	0.04	9.21	0.45	0.196*
Salicaceae	<i>Populus nigra</i> L.	27	0.57	42.67	0.02	7.41	1.06	0.394*
	<i>Salix alba</i> L.	46	0.96	44.86	0.03	12.07	0.73	0.140*
	<i>Salix caprea</i> L.	46	0.64	46.35	0.02	12.98	0.67	0.216*
Tiliaceae	<i>Tilia cordata</i> Mill.	27	0.93	46.07	0.09	11.79	1.23	0.204*
	<i>Tilia platyphyllos</i> Scop.	18	0.20	47.35	0.09	11.03	0.94	0.172*
Ulmaceae	<i>Ulmus glabra</i> Huds.	10	0.96	49.68	0.13	5.61	3.83	0.067*
	<i>Ulmus minor</i> Mill.	17	0.58	47.53	0.09	5.61	2.17	0.456*

**Figure S1** Correlations between litter traits (lignin, N and P concentrations) with p and r values (Pearson correlations)



## Supplementary Material 2

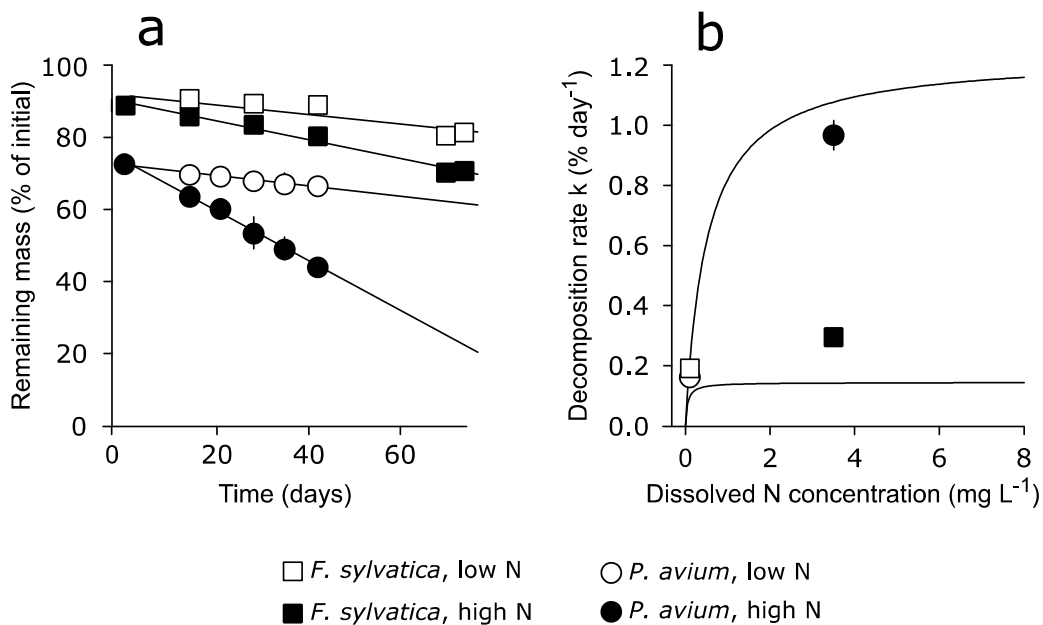
**Table S2** Relative proportions ( $\% \pm \text{SD}$ ,  $n=10$ ) of the abundance of aquatic hyphomycete species found in 2 mL of inoculum, and number of samples (out of 10) in which each species was observed.

Species	Abundance	# of samples
<i>Tetrachaetum elegans</i> Ingold	$41.81 \pm 6.04$	10
<i>Flagellospora curvula</i> Ingold	$15.81 \pm 2.27$	10
<i>Crucella subtilis</i> Marvanová & Suberkropp	$12.3 \pm 2.24$	10
<i>Tricladium chaetocladium</i> Ingold	$7.74 \pm 1.97$	10
<i>Alatospora acuminata</i> Ingold	$7.12 \pm 1.88$	10
<i>Articulospora tetracladia</i> Ingold	$5.52 \pm 2.30$	10
<i>Goniopila monticola</i> (Dyko) Marvanová & Descals	$1.99 \pm 0.84$	10
<i>Taeniospora gracilis</i> Marvanová	$1.68 \pm 0.82$	10
<i>Clavatospora longibrachiata</i> (Ingold) Marvanová & Nilsson	$1.49 \pm 0.56$	10
<i>Anguillospora filiformis</i> Greathead	$0.95 \pm 0.60$	10
<i>Neonectria lugdunensis</i> (Saccardo & Therry) L. Lombard & Crous	$0.75 \pm 0.48$	10
<i>Anguillospora longissima</i> Sacc. & Syd.) Ingold	$0.73 \pm 0.39$	10
<i>Fontanospora eccentrica</i> (Petersen) Dyko	$0.67 \pm 0.57$	10
<i>Stenoclatrella neglecta</i> (Marvanová & Descals) Marvanová & Descals	$0.53 \pm 0.64$	7
<i>Clavariopsis aquatica</i> De Wild.	$0.33 \pm 0.39$	10
<i>Alatospora pulchella</i> Marvanová	$0.29 \pm 0.45$	8
<i>Culicidospora aquatica</i> Petersen	$0.14 \pm 0.22$	10
<i>Geniculospora inflata</i> (Ingold) S. Nilsson ex Marvanová & Nilsson	$0.10 \pm 0.20$	3
<i>Lunulospora curvula</i> Ingold	$0.03 \pm 0.08$	2
<i>Tricladium splendens</i> Ingold	$0.01 \pm 0.01$	7
<i>Heliscella stellata</i> (Ingold & Cox) Marvanová & Nilsson	< 0.01	2
<i>Anguillospora crassa</i> Ingold	< 0.01	2
<i>Mycofalcella calcarata</i> Marvanová, Om-Kalth. & Webster	< 0.01	1

### Supplementary Material 3

This supplementary material displays the results of a preliminary experiment carried out in conditions similar to those of the main experiment but on 2 litter species with contrasting quality (*Prunus avium* and *Fagus sylvatica*). Two mineral solutions were identical to the ones used in the main experiment, but with N concentration of 0.105 and 3.5 mg N L<sup>-1</sup> for the N-poor and the N-rich solution, respectively. Because this preliminary experiment was carried out at a different time of the year, the fungal inoculum, though obtained using the same procedure as the main experiment, probably contained different fungal species.

This experiment primarily aimed at testing the relevance of microplates to study litter decomposition, and at determining the shape of leaf mass loss dynamics in these experimental conditions. In this supplementary material, we also compare the results of this experiment with the main experiment, which gives support to the robustness of our main conclusions with an experiment carried out in similar conditions, but with a different fungal inoculum.



**Fig. S3** (a) Decomposition dynamics of 2 litter species in N-limiting and N-rich treatments (mean  $\pm$  SD,  $n=3$ ). Lines are the linear regressions used to calculate  $k$  values. (b) Effect of dissolved N concentration on the average decomposition rate of these two species. The upper and lower lines are the Michaelis-Menten models from the main experiment for *P. avium* (upper line) and *F. sylvatica* (lower line)