Faster than expected: Release of nitrogen and phosphorus from decomposing wood

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Summary

- Deadwood represents globally important carbon, nitrogen, and phosphorus pools. Current wood nutrient dynamics models are extensions of those developed for leaf litter decomposition. However, tissue structure and dominant decomposers differ between deadwood and litter, and recent evidence suggests that decomposer stoichiometry in combination with litter quality may affect nutrient release.

- We quantified decomposition and release of carbon and nutrients from wood for two stem sizes of 22 tree species in a phosphorus-limited temperate forest near Sydney, Australia, and compared these to estimates from leaf litter literature.

- Following theory, nitrogen and phosphorus accumulated during early decomposition, but began to decline earlier than expected from work on leaves. Deadwood converged on higher carbon:nitrogen (50) and nitrogen:phosphorus (80) ratios than in leaf litter studies. Carbon:nitrogen at which nitrogen was released was higher in large stems (~135) than small stems (~95); both being higher than leaf litter.

- Drawing from the literature, these differences in nitrogen and phosphorus dynamics may be due to the identity of wood decomposers. Carbon:nitrogen of wood decomposers is higher than mean carbon:nitrogen of leaf litter decomposers, and this difference in stoichiometry may have important flow-on effects for nutrient cycles in forests.

Keywords

Decomposition, Deadwood, Nutrient cycling, Microbes, Stoichiometry, Temperate forest

Introduction

Deadwood represents up to 8% of forest carbon (C) stocks and is a crucial component of nutrient stores (Harmon *et al.*, 1986; Pan *et al.*, 2011). Deadwood has low concentrations of nitrogen (N) and phosphorus (P) compared with other dead vegetation components, such as leaf litter, making wood a low quality resource for decomposers (Laiho & Prescott, 2004). However, some decomposing microbes, specifically wood saprotrophic fungi, have adapted to exploit deadwood despite its low quality, promoting nutrient cycling within forests (Lindahl *et al.*, 2002). Compared with leaf litter, nutrients stored within deadwood exhibit a slower turnover due to wood's slower decomposition rates providing a more long-lasting source of nutrients to the ecosystem. Currently, most knowledge of nutrient cycling within dead vegetation pools comes from leaf litter (Parton *et al.*, 2007; Manzoni *et al.*, 2010). However, because of the large biomass and unique structural properties and contributions to the nutrient and C cycles as compared to leaf litter, it is important to understand the factors that determine the rate at which C and nutrients are released from deadwood.

Previous studies showed that deadwood retains, and even increases, nutrient concentrations such as N and P during the decomposition process (Harmon *et al.*, 1986; Herrmann & Prescott, 2008; Palviainen *et al.*, 2008; Meriem *et al.*, 2016; Smyth *et al.*, 2016; Castillo *et al.*, 2023). Such nutrient accumulation is known as immobilisation and is primarily driven by the foraging behaviour of microbes, which have adapted different strategies to efficiently process C while retaining nutrients (Manzoni *et al.*, 2021). These strategies include the alteration of C use efficiency (Manzoni *et al.*, 2008), import of nutrients from the surrounding environment (Wells & Boddy, 1995) and increased retention time of elements within microbes after senescence (Spohn & Widdig, 2017). The nutrients accumulate and are immobilised within the deadwood as C concentrations decrease until a certain threshold of nutrient:C ratio is reached. Once this threshold is met, microbes and their substrates are at a stoichiometric balance meaning that nutrients are no longer limiting the growth of microbes and can be exported to other mycelium or released to the soil (Zechmeister-Boltenstern *et al.*, 2015). In addition, other mechanisms, although less influential, such as N fixation or physico-chemical processes including wicking can contribute to the accumulation of N (Smyth *et al.*, 2016). As N concentrations increase while C concentrations decrease, the C:N ratio will also decrease during the decomposition process until it stabilises ~20 (Manzoni *et al.*, 2010).

Deadwood resources vary significantly in nutrient concentrations depending on the age and species of a fallen tree (Harmon *et al.*, 1986), which may lead to differences in the amount of time nutrients are immobilised within the resource leading to different lag times of when there are net releases of nutrients. Theory developed for leaf litter decomposition suggests that the initial nutrient concentration of litter is the main determinant for an observed lag in the release of nutrients from a substrate (Parton *et al.*, 2007; Manzoni *et al.*, 2010, 2021). Nutrients within leaf litter with low nutrient availability tend to be released later in the decomposition process than from more nutrient rich litter. In concurrence, microbes have developed adaptations such as a reduction in C use efficiency (Manzoni *et al.*, 2008) and extraction of additional nutrients from the surrounding soil to cope with low nutrient availability (Frey *et al.*, 2000; Parton *et al.*, 2007; Manzoni *et al.*, 2021). Studies found that these adaptations lead to critical changes in the stoichiometric balance of litter at which N is released ($Crit_{CN}$), which tends to increase as initial N increases (Manzoni *et al.*, 2008; Ågren *et al.*, 2013). For litter, $Crit_{CN}$ is typically between 30 and 50 (Parton *et al.*, 2007) but this value is likely higher in deadwood due to its higher initial C:N ratios (Manzoni *et al.*, 2010; Smyth *et al.*, 2016). While these processes are fairly well understood within leaf litter, the factors controlling the accumulation and release patterns in N and P for deadwood are not.

In translating nutrient cycling theory for leaf litter to deadwood, besides differences in their stoichiometry, the other main differences between leaf litter and deadwood are size and geometric structure. Contrary to litter which tends to have a two-dimensional structure and low variation in surface area:volume, deadwood is three-dimensional and variable in volume depending on the diameter and length of the branches and stems falling to the ground. Different sized wood may influence decomposition rates and C loss; for instance, smaller diameter stems decompose faster (Mackensen *et al.*, 2003; Weedon *et al.*, 2009; Hu *et al.*, 2018; Lee *et al.*, 2022). Such differences are thought to be because of the higher surface area-to-volume ratios and higher accessible nutrient concentrations in small as compared to large stems, which allows for a faster and more efficient colonisation of the substrate by microbes (Cornwell *et al.*, 2009). In light of these expectations, we predicted a faster colonisation process and a higher proportional nutrient concentration in small stems, which could lead these stems to immobilise nutrients for shorter periods than larger stems of the same species.

The assimilation and release patterns of different nutrients such as N and P may also depend on how saprotrophs exploit the resources and availability of these resources in the surrounding environment. For instance, N is expelled from organisms and reabsorbed as it is

crucial for the functioning of extracellular enzymes (Walker & White, 2017). In contrast, P is mostly retained inside saprotrophic organisms (Beever & Burns, 1981). We predicted P to be released earlier in the decomposition process than N as it is more efficiently retained by the saprotrophs and not as dependent on exterior stoichiometry of the resource to be reabsorbed. N-limited or P-limited ecosystems may also lead to different strategies in how organisms process these resources. As the concentration of P in the surrounding environment is lower, we also predicted that it would be exported earlier from deadwood as there is higher competition for P and other organisms may be actively syphoning the nutrients (Zechmeister-Boltenstern *et al.*, 2015).

To better understand the dynamics of nutrients within deadwood through time, we used a decomposition experiment including 22 woody species in a sclerophyll woodland in Australia where P limits productivity (Ellsworth *et al.*, 2017). The nutrient concentrations of deadwood for each species were measured at five time points over a five-year window to accurately track their changes through time. Following theory based on litter decomposition (Parton *et al.*, 2007; Manzoni *et al.*, 2010) and previous studies looking at changes in nutrients through time for deadwood (Fig. 1), we formulated four hypotheses:

- H_1: We expected nutrient (N and P) concentrations in deadwood to initially increase until a certain mass remaining threshold is reached at which point a net nutrient release (Net_N for N and Net_P for P) will occur. We expected Net_N and Net_P of wood to be similar to that of low-quality leaf litter, i.e. ~40% of mass remaining. We also expected N to be released at a C:N ratio above 50 and P to be released before N.
- H_2: We expected deadwood with higher initial N and P to release nutrients earlier in the decomposition process (i.e., at higher mass remaining) and at a lower $Crit_{CN}$.
- H_3: We expected smaller size wood to have a higher N and P concentration and release nutrients earlier in the decomposition process while N would be released at a lower $Crit_{CN}$.
- H_4: Given the P limitation of our site, we expected N:P ratios of stems to be >30 (Zechmeister-Boltenstern *et al.*, 2015) and decrease during the decomposition process up to that of saprotrophic fungi at ~13 (Zhang & Elser, 2017). We expected C:N ratios to decrease until a threshold of ~20 (Manzoni *et al.*, 2010) as fungi create a favourable environment for C assimilation throughout the decomposition process.

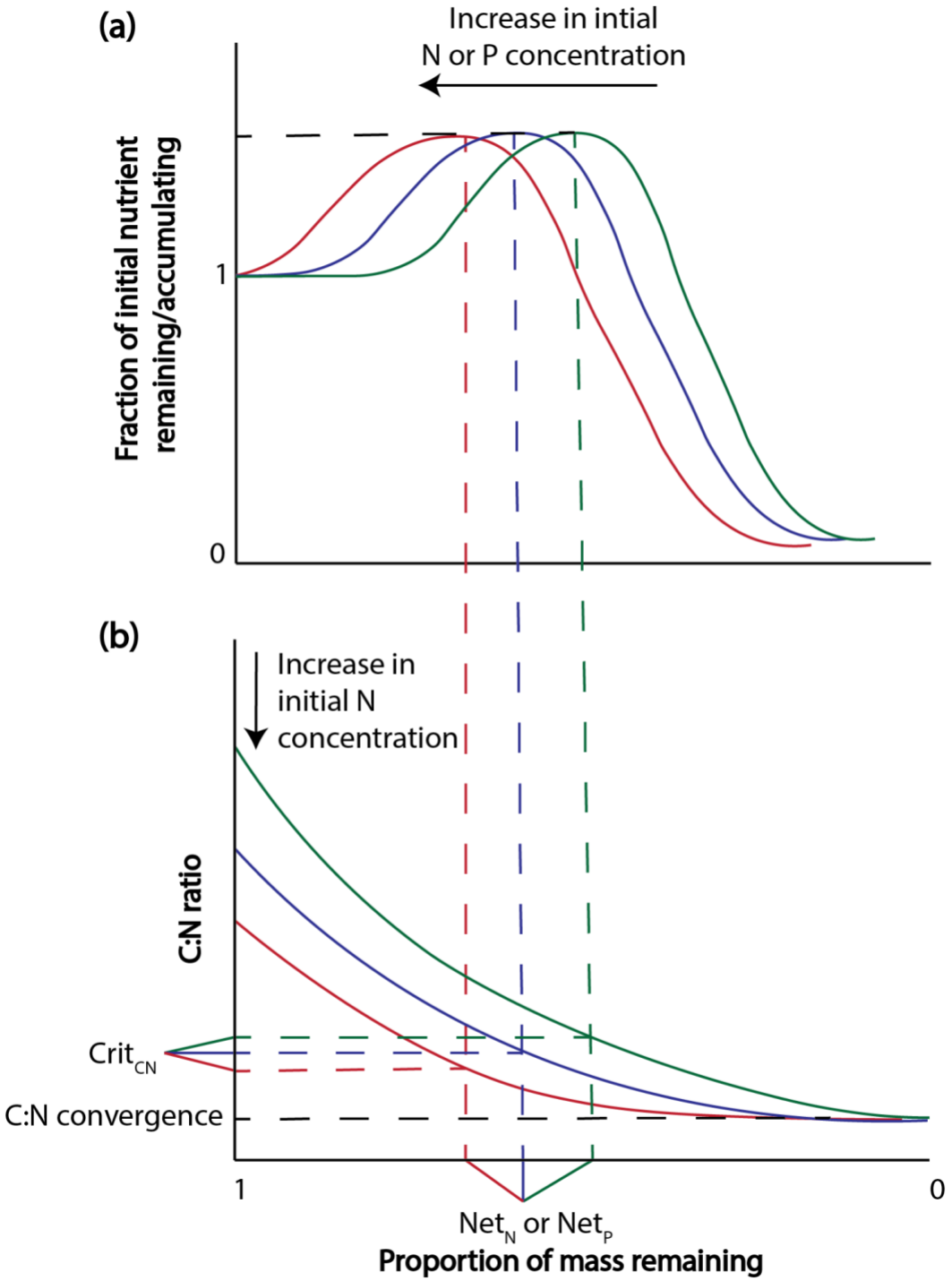


Figure 1 - (a) Nutrient release curves and (b) C:N ratio of wood against proportion of mass remaining. Each coloured line represents changes in (a) fraction of initial nutrient remaining/accumulating and (b) C:N ratios of a species against proportion of mass remaining with species ranging in initial N or P concentration (green < blue < red). The intersection of the peak of the nutrient release curve with the proportion mass remaining represents the mass remaining threshold when there is a net release of the nutrient (Net_N for N and Net_P for P). The intersection of Net_N with the corresponding C:N ratio corresponds to $Crit_{CN}$.

Materials and Methods

Study design

We measured the decomposition of wood from 22 tree species in Cumberland Plain Woodland on the Western Sydney University Hawkesbury campus (33°37′13″S 150°44′16″E). The tree species were selected from three sites including Agnes Banks Nature Reserve, Castlereagh Nature Reserve and the Cumberland Plain SuperSite of the Terrestrial Ecosystem Research Network. More information regarding these sites and the selected species can be found in Lee *et al.* (2019) and Lee *et al.* (2022). All species are found within sclerophyll woodlands with mean annual rainfall of 728.1 mm and mean annual temperatures of 24.3 (max) to 11.1 (min) °C. For each species, we collected a live stem from at least 3 individuals belonging to a smaller (1–2 cm diameter) size class. For 12 of these species, we were also able to collect a live stem from at least three individuals from a larger (5-9 cm diameter) size class. The species for which we did not have a larger size class do not naturally produce stems of these sizes. For both size classes, each stem was cut into individual pieces of wood, each 10 cm long.

At the start of the decomposition study, 30 randomly selected stems for each combination of species and size class were set out in the field on 4-5 July 2013. The stems were set out across three 7 m x 1 m plots, each separated by 1 m, in a completely randomised design. We raked the plots before the deployment of the wood stems to remove litter and coarse woody debris. We checked that there were no trees or woody shrubs within 1 m of any plot. Within each plot, pieces were set out at a density of 50 per m^2 with five woody stems placed lengthwise in rows parallel to the short edge of the plot. We left roughly 10 cm of space between the ends of each stem. The stem placement was completely randomised. Finally, we used aluminium tags fastened with plastic cable ties to each stem, and these were fastened to the ground with a steel pin looping around the stem (Lee *et al.*, 2022). Up to six randomly selected pieces of wood from each species were collected at five sampling points (February 2014, August 2014, August 2015, August 2016 and June 2018) for the large size class and four sampling occasions (all dates except June 2018) for the small size class. Particularly late in the experiment, we were unable to collect six pieces due to the wood being too decomposed to recover. At each time point, the stems were collected from the field and placed in an oven at 105°C for five days at which point dry mass was obtained. We then calculated the proportion of mass remaining for each piece of wood (equation 1).

$$x(t) = \frac{m(t)}{m(t_1)} \quad (1)$$

Where *m* represents the dry mass at time *t* and initial mass t_1.

Wood chemistry

At the start of the experiment, elemental concentration measurements (for C, N and P) were made on 1-3 undeployed pieces of wood coming from the same branches and individuals as used in the experiment. These were collected for each combination of species and size class where possible (Lee *et al.*, 2022). The elemental concentrations were then measured on three individual pieces of wood per harvest, except for the final harvest after five years where measurements were taken on up to six individual pieces of wood, with the number depending on how many could be recovered.

For each piece of wood, 150 mg of dried and milled wood was analysed for elemental concentrations. C and N were determined using a TruMac CN Macro analyzer (Leco, St. Joseph, Michigan, USA) and P was measured using an Epsilon 3X X-ray fluorescence spectrometer from PANalytical (Sydney, Australia). In total, we obtained 543 samples with N and C measurements and 481 samples with P measurements.

Analysis

To look at the differences in initial N and P concentration of small and large stems, we used a linear mixed effects model. We averaged N and P concentrations for each combination of species and size class from the undeployed pieces of wood and used these as a response variable. Size class was used as a fixed explanatory variable and species as a random variable to account for variability in responses across species.

The speed of decomposition for each species and size class combination has been examined in Lee *et al.* (2022). Decomposition rates ranged between 1.7 to 4.8 years at 50% mass remaining in small stems, while ranging from 2.2 to 6 years at 50% mass remaining in large stems. Here we focused on changes in chemistry through the decomposition process. To quantify such changes, we calculated the fraction of each element remaining in relation to the mass remaining of the wood stem (equation 2).

$$f(t) = \frac{(n(t) * x(t))}{(n(t_1) * x(t_1))} \quad (2)$$

Where *n* is the concentration of the element at time *t* and *x* is the proportion of initial mass remaining of the wood stem at time *t* or t_1. To quantify the lag in release of each element through the decomposition process of wood, we used the equation in Parton *et al.* (2007), (equation 3).

$$f(x(t), a, b) = \frac{(x(t)*100)/b}{\sqrt{(2*a*(x(t)*100)/b)^2 + (1-((x(t)*100)/b)^2)^2}} \quad (3)$$

where *a* is the control factor for the peak value of the function and *b* is the location on *x* for the peak value of the function. Using the package *litterfitter* (Cornwell & Weedon, 2014) in R (*v 4.2.1*), we computed the optimal values of a and b which generates the function with the best fit to the fraction of the element remaining against mass remaining. Net_N and Net_P were calculated as b divided by 100 to standardise these to proportions (equation 4).

$$Net_N \mid Net_P = \frac{b}{100} \quad (4)$$

We extracted Net_N or Net_P for each species and size class combination. Using the *glmmPQL* package, we ran generalised linear mixed effects models with Net_N or Net_P as response variables against size and initial nutrient concentration as fixed factors. In the case of the model with Net_N as a response, we multiplied initial N concentration by 10 to model changes in probability with every 0.1% change in initial N. This was done given the initial N content of deadwood ranged between 0.08 and 0.8. Additionally, for models using Net_N or Net_P, we scaled the response around the mean to obtain a mean-centred intercept. Such intercepts correspond to the modelled probability for the mean values of initial N and P concentration, respectively. Species was used as a random effect to account for variability in responses across species. We applied a quasibinomial distribution to the model as Net_N or Net_P are proportions which are found within a value of 0 and 1.

To compute the critical C:N ratio at which N was released, we extracted the mass remaining at which N was released (Net_N) for each combination of species and size class. We then quantified the change in C:N ratio throughout the decomposition process assuming an exponential increase in the ratio against proportion mass remaining (i.e. an exponential decrease throughout the decomposition process), (equation 5).

$$f(x) = r^x + i \quad (5)$$

Where r controls the rate of increase in C:N and *i* determines the point of convergence of C:N at 0% mass remaining (i.e. the output of the equation at $x = 1$). We generated the line of best fit using the *litterfitter* package which extracted the optimal *r* and *i* values for each species and size class combination from which we computed the C:N ratio corresponding to $Net_N(Crit_{CN})$ as in equation 6.

$$Crit_{CN} = c^{NetN} + i \quad (6)$$

For each piece of wood, we also calculated the C:N and N:P ratios. We then ran linear mixed effects models to look at trends in C:N and N:P ratios through time against proportion mass remaining and size of stems as explanatory variables. Similar to the other models, species were used as a random variable. The ratios were log-transformed to account for their right skew towards lower C:N and N:P values.

Results

Characteristics of wood

Smaller stems had a higher initial N concentration than larger stems (Estimate (Small) = 0.13, 95% CI [0.08-0.18], $p < 0.001$) while there was no difference in initial P concentration between stem sizes (Estimate (Small) = 22.09, 95% CI [-27.2-71.43], $p = 0.37$), (Fig. 2).

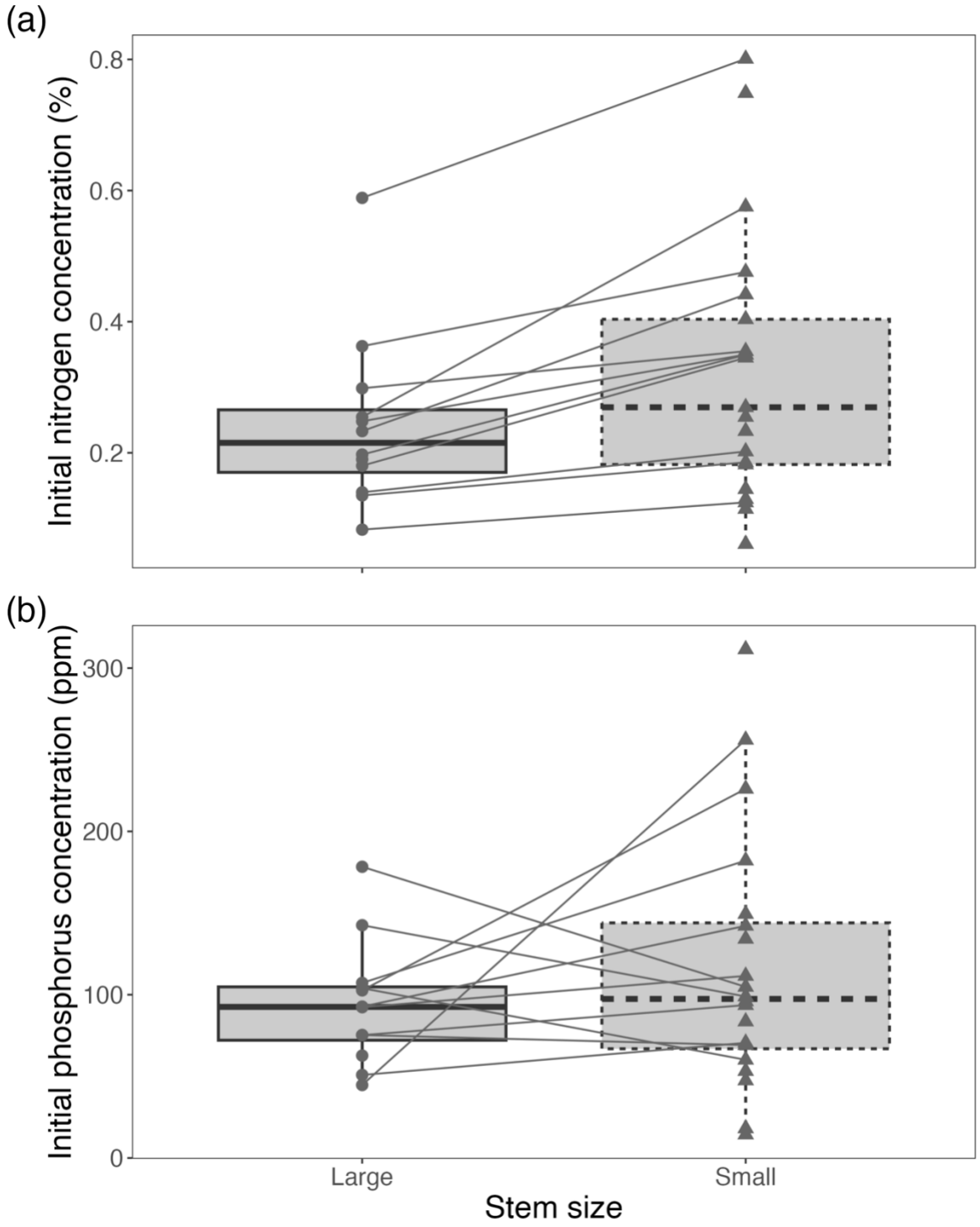


Figure 2 – (a) Initial N concentration and (b) initial P concentration of large (solid contour line) and small (dashed contour line) wood stems. Each point represents a species average and lines link averages for the same species of different sizes.

Nutrient release patterns and stoichiometry

The fraction of N and P increased in the first stages of the decomposition for most but not all wood species (Fig. S1, Fig. S2, Fig. S3, Fig. S4). N and P started to be released at a mean (± SD) of 0.68 (± 0.23) and 0.81 (± 0.2) of proportion mass remaining, respectively.

Wood species with a lower initial N concentration released N at a lower mass remaining (Table 1, Fig. 3a). Similarly, wood species with a lower P concentration released P at a lower mass remaining (Table 1, Fig. 3b). However, stem size was not observed to influence the proportion of mass remaining at which either N or P were released (Table 1).

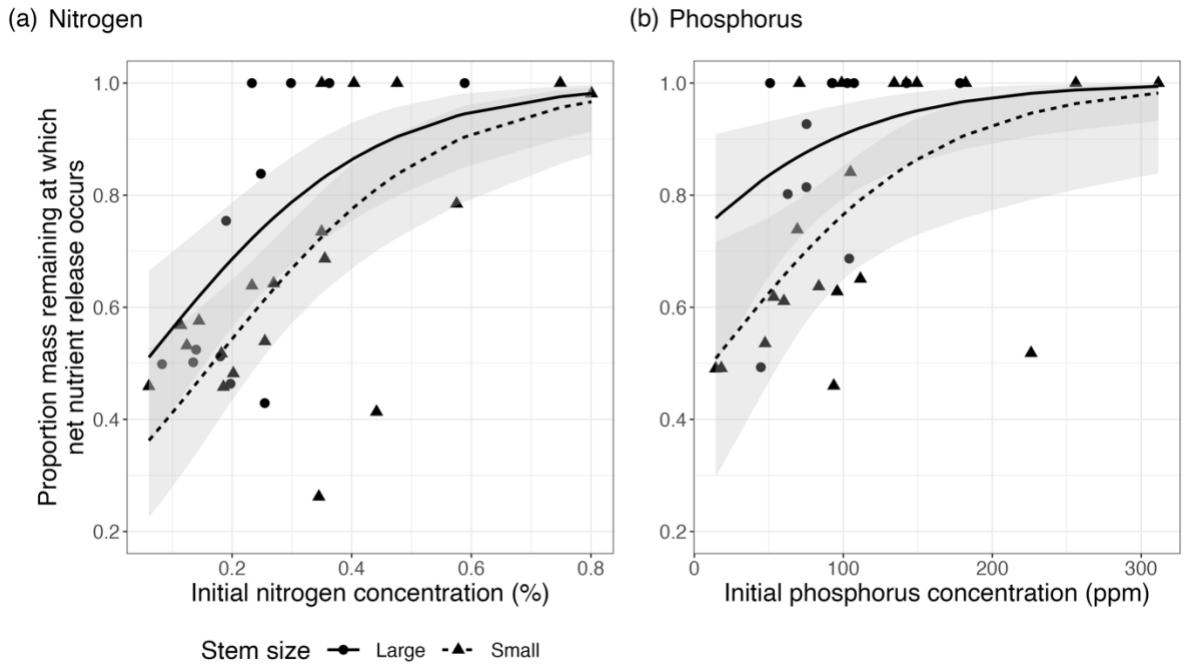


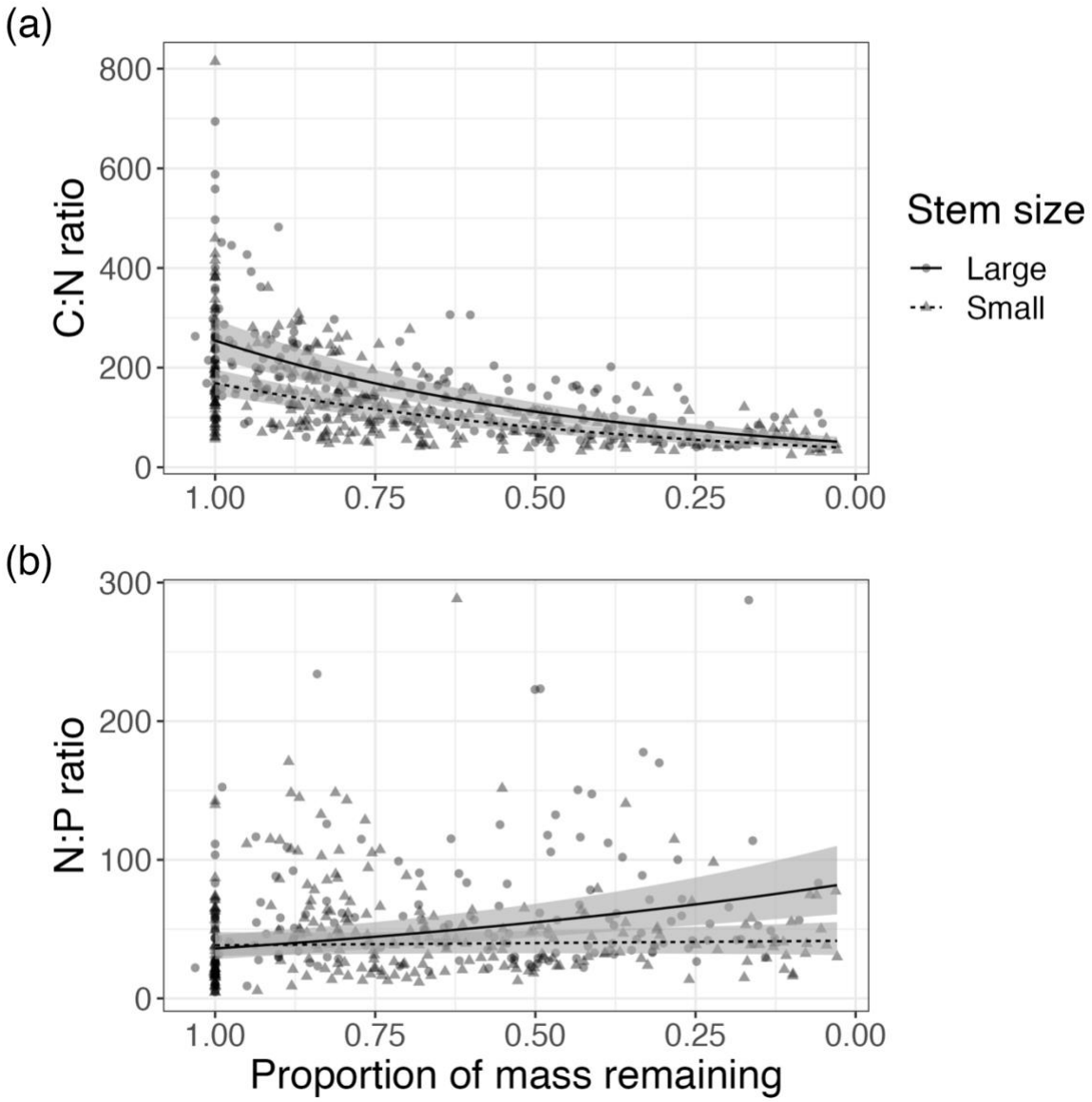
Figure 3 - Proportion mass remaining at which (a) N (Net_N) and (b) P (Net_P) were released from wood against initial N or P concentration respectively in large (solid line) and small (dashed line) stems.

Table 1 - Linear mixed effect model output of proportion mass remaining at which N (Net_N) or P (Net_P) started being released against initial N or P concentration, respectively, and size of stem. Species of wood were specified as a random factor. P-values in bold were significant at >0.05. To account for the low variation in nutrient concentration among wood species (ranging from 0.08 to 0.8) and calculate the probability for every increase in 0.1% initial N concentration, initial N concentration was multiplied by 10 and scaled around the mean to obtain a mean-centred intercept. Initial P concentration was also scaled around the mean. The mean-centred intercepts correspond to the modelled probability for the mean values of initial N and P concentration, respectively.

Response	*Predictors*	*Probability*	*CI*	*p*
Net_N	*(Mean-centred intercept)*	0.79	0.67 – 0.87	**<0.001**
	*Initial N concentration*10 (%)*	0.72	0.60 – 0.82	**0.004**
	Size [Small]	0.35	0.2 – 0.54	0.125
Net_P	*(Mean-centred intercept)*	0.92	0.80 – 0.97	**<0.001**
	Initial P concentration (ppm)	0.71	0.53 – 0.84	**0.036**
	Size [Small]	0.25	0.09 – 0.53	0.086

Notes: Random effect outputs for Net_N: σ^2 = 3.29, $\tau_{00\ species}$ = 0.03, ICC = 0.01, $N_{species}$ = 21. Observations = 32, R^2 = 0.21. Random effect outputs for Net_P: σ^2 = 3.29, $\tau_{00\ species}$ = 0, ICC = 0, $N_{species}$ = 21. Observations = 32, R^2 = 0.22.

C:N ratios slowly decreased throughout the decomposition process from a high of ~250 for large stems and ~180 for small stems at the start of the decomposition process to a value of ~50 at the end regardless of size (Fig. 4a, $p < 0.001$). On the contrary, N:P ratios were ~40 and similar for both sized stems at the start of the decomposition process. However, N:P ratios increased with decomposition to ~80 for large stems while staying similar throughout the decomposition process for small stems (Fig. 4b, $p < 0{,}001$). N was released at an average $Crit_{CN}$ of 110. $Crit_{CN}$ was higher in large stems (~135) than small stems (~95) but was not influenced by the initial concentration of N (Fig. 5, Table 2).



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339 Figure 4 – (a) C:N ratio and (b) N:P of wood stems against proportion mass remaining in

340 large (solid) and small (dashed) stems.

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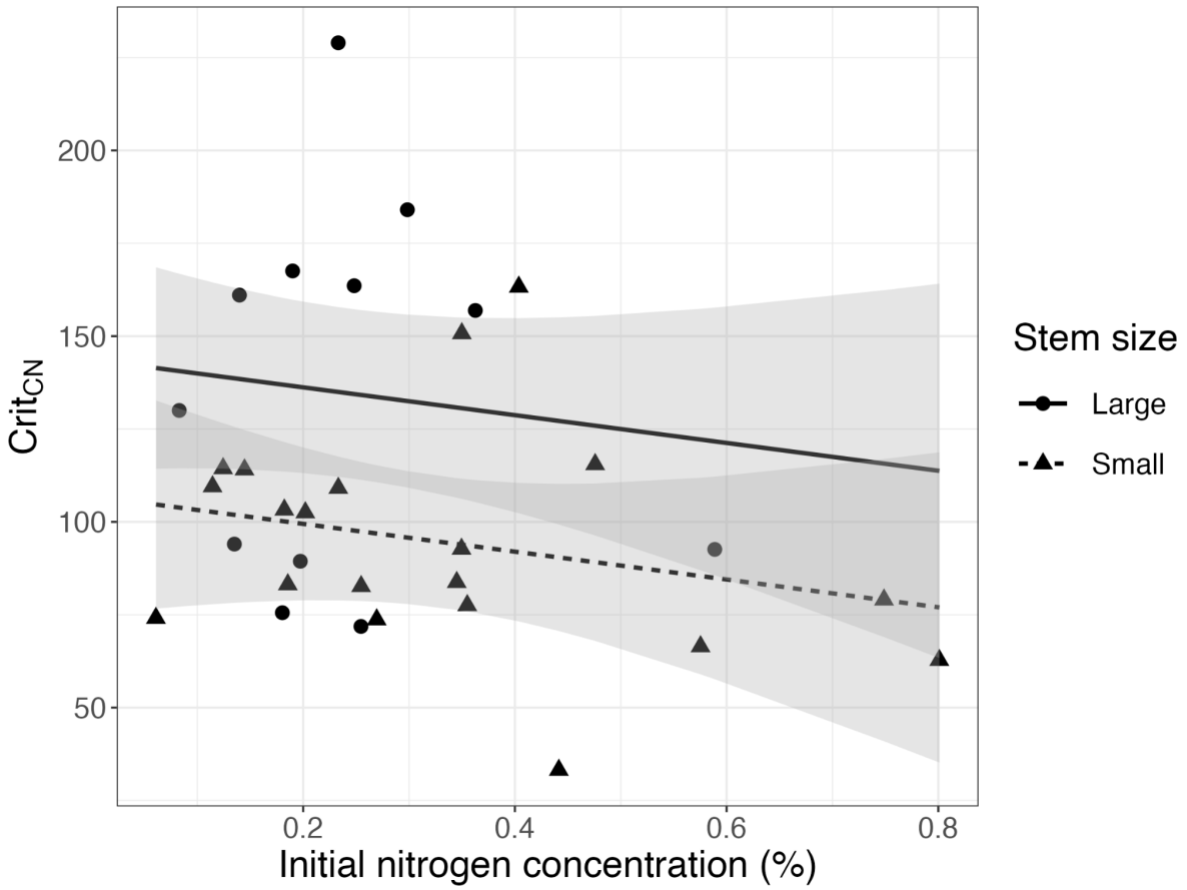

Figure 5 - C:N ratio at which N started being released ($Crit_{CN}$) against initial N concentration in large (solid line) and small (dashed line) stems.

Table 2 - Linear mixed effect model output of C:N ratio at which N is released against size of stem and initial N concentration. Species of wood were specified as a random factor. P-values in bold are considered significant.

		$Crit_{CN}$	
Predictors	*Estimates*	*CI*	*p*
(Intercept)	143.70	113.68 – 173.73	**<0.001**
Size [Small]	-36.76	-66.48 – -7.04	**0.017**
Initial N concentration (%)	-37.42	-117.86 – 43.02	0.348

Notes: Random effect outputs: σ^2 = 1483.83, $\tau_{00\ species}$ = 0, $N_{species}$ = 21. Observations = 32, R^2 = 0.23.

Discussion

We investigated the factors influencing the accumulation and release of nutrients from decomposing wood during the decomposition process. Given the dearth of similar studies in wood, our predictions were based on studies on leaf litter and we found that these were only partially supported (Table 3). In line with hypothesis 1, nutrients accumulated until a certain mass remaining threshold was reached; however, nutrients were released earlier in the decomposition process for wood compared to even low quality litter (comparison with Moore *et al.*, (2006) and Parton *et al.*, (2007)), with deadwood from several species showing no lag in release. In accordance with hypothesis 2, the release of N (Net_N) and P (Net_P) occurred at a lower mass remaining for stems with lower initial N and initial P concentrations. However, contrary to litter studies (Manzoni *et al.*, 2008; Ågren *et al.*, 2013), $Crit_{CN}$ did not decrease with initial N. Contrary to our expectations from hypothesis 3, stem size did not influence mass remaining at which N and P were released from the wood, although stems with a larger size had a higher $Crit_{CN}$. Finally, while general trends in C:N and N:P ratios agreed with our expectations under hypothesis 4, C:N and N:P converged to a higher (~50 or 80, respectively) value than expected based on average fungal stoichiometry and previous studies on leaf litter (Moore *et al.*, 2006; Manzoni *et al.*, 2010). The unique properties of wood, which attract specially adapted organisms (Wijas *et al.*, 2024), likely lead to singular and surprisingly dynamic properties in the release of nutrients within forest ecosystems. Throughout these next sections, we discuss our results in the context of the state of the field, largely shaped by studies carried out on leaf litter.

390 Table 3 - Different characteristics in the release of N and P from leaf litter and deadwood.
391 Findings for leaf litter from the literature as noted and findings for deadwood from this study.

Hypothesis	Characteristic	Leaf litter	Deadwood
H_1	Net_N	40-50% mass remaining (forest data from Parton *et al.*, (2007))	68% mass remaining
H_1	Net_P	57% mass remaining (Tamarack needles in Moore *et al.*, (2006))	81% mass remaining
H_1	$Crit_{CN}$	~40 (Parton *et al.*, 2007)	110
H_2, H_3	Decrease in Net_N with initial N concentration	Yes (Parton *et al.*, 2007; Manzoni *et al.*, 2010)	Yes (72% probability of increase in Net_N for every 0.1% increase in initial N)
H_2, H_3	Decrease in Net_P with initial P concentration	Yes (Parton *et al.*, 2007; Manzoni *et al.*, 2010)	Yes (71% probability of increase in Net_P for every increase in 1 ppm of initial P)
H_2, H_3	Decrease in $Crit_{CN}$ with increased initial N content	Yes (Ågren *et al.*, 2013)	No, but smaller stems with a higher N concentration had a lower $Crit_{CN}$ compared with larger stems
H_4	C:N convergence	20 (Manzoni *et al.*, 2010) 30 (Moore *et al.*, 2006)	50 (regardless of stem size)
H_4	N:P convergence	20 (Manzoni *et al.*, 2010) 16 (Moore *et al.*, 2006)	40 in small stems, 80 in large stems

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394 *Variation in immobilisation and release: What may be driving it?*
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We found that N and P increased in concentration until an earlier threshold of mass remaining, 68% and 81% mass remaining, respectively, compared to previous work on leaf litter (Moore *et al.*, 2006; Parton *et al.*, 2007). These results suggest that export of N and P balances and then exceeds import at an earlier stage in the decomposition trajectory. These results appear surprising: why does wood with a lower initial N than leaf litter release N sooner in the decomposition trajectory compared to leaf litter? One possible explanation for this incongruity is the recognition that different decomposers break down leaf litter and deadwood and the stoichiometric needs of them may be different, i.e., the mean C:N of wood decomposers is much higher than the mean C:N of leaf litter decomposers. For instance, the C:N ratios at which white rot fungi, which are adapted to degrade lignin in deadwood, function optimally are estimated to be between 160 and 400 (Eriksson *et al.*, 1980). Under this explanation convergence on decomposer C:N occurs at a relatively higher mass remaining of deadwood, leading to the surprising release of N (and P) at a high mass remaining. It is worth noting two things in this context. First, the decomposition process of wood is significantly slower than that of leaf litter (Pietsch *et al.*, 2014), therefore the release dynamics are occurring at different time scales (across months for leaf litter compared to years to decades for deadwood). Second, N and P may not be externally available during the "release" phase; a portion of these could be exported to belowground fungal mycelium in a similar way that import occurs early in the decomposition process.

Although our study was conducted in a P-limited ecosystem, we found that P was released at a higher mass remaining of deadwood than N. This finding may be indicative of the dominance of fungi within the decomposition of our substrates given their lower reliance on P and their higher tolerance to P-limitations than other saprotrophs such as bacteria (Güsewell & Gessner, 2009). Unlike N, P is not required for the functioning of extracellular enzymes meaning these can be retained within the cells of fungi (Walker & White, 2017). Through the transport of P within their cells, fungi can target specific areas to improve stoichiometric balance and favour the intake of further nutrients (Beever & Burns, 1981). Additionally, the low availability of P within the soil may increase competition for P and lead to an early export through the mycelial networks of fungi rather than imports from the soil (Boddy, 1999). In contrast, the higher availability of N allows the element to accumulate for longer periods of time. Increases in N:P ratios during the decomposition process, especially for larger stems, further suggests that higher N is required for the functioning of microbes compared with P, whether P is being transported out or N is being imported during decomposition. An increase in N:P ratios in large stems during the decomposition process also goes against what is generally assumed for leaf litter (Manzoni *et al.*, 2010) in which it stays unchanged, as we found within small stems.

Tree species with higher initial wood N and P concentrations released their respective nutrients at a higher proportion mass remaining. These results were similar to those for leaf litter from Parton *et al.* (2007) and Manzoni *et al.* (2010) and are consistent with models showing that stoichiometric balance is obtained at a higher mass remaining in tissue with higher initial nutrients. Contrary to leaf litter results, however, $Crit_{CN}$ did not decrease as initial N increased (Manzoni *et al.*, 2008, 2010). Microbial communities within deadwood stems with low nutrient concentrations attained a similar C:N ratio before the net release of N to those of microbial communities with high nutrient concentrations. Our finding suggests that stems with low N concentrations either accumulated larger amounts of N and/or released more C (e.g., via respiration) than stems with high N concentration before they began releasing N. Stems with lower N concentration could therefore have outsized contributions to N or C cycling in forest ecosystems.

Deadwood size: When does it matter?

Small stems did not show a release of N or P at higher mass remaining than large stems although they contained higher concentrations of N (although P did not differ between stem sizes; Fig. 2 and Fig. 3). It may seem surprising that higher initial N concentrations did not lead to earlier release of N in the decomposition process; however, $Crit_{CN}$ was also lower in these stems. Integrating these two results suggests that the higher $Crit_{CN}$ in larger stems may lead to a similar Net_N between small and large stems compensating for the initial difference in N concentrations. The size of the substrate therefore may have modulated how microbes exploited their resources as well as which microbe species could exploit the resource. Indeed, it is well documented that deadwood with different diameters support different microbial communities (Juutilainen *et al.*, 2011; Krah *et al.*, 2018). Our results suggest decomposition was more nuanced than initially expected, depending on the relative initial and changing concentrations of multiple elements and may mean we need to consider multiple metrics, e.g., both $Crit_{CN}$ and initial N concentrations, when trying to predict the cycling of nutrients from deadwood within forest ecosystems.

Overall, as decomposition progressed, the stoichiometry of wood stems changed, although these effects varied depending on the size of the stems. Similar to studies on litter decomposition, we showed that C:N decreased as decomposition progressed (Moore *et al.*, 2006; Manzoni *et al.*, 2010), which is most probably due to a combination of N immobilisation and C respiration. However, while previous estimates based mainly on leaf litter decomposition suggest that C:N ratios converged ~15-20, we found that C:N ratios

converged ~50 in our study. Interestingly, C:N ratios decreased at a higher rate within large stems compared with small stems. Given N release occurred at similar stages of decomposition between small and large stems, this suggests C was respired at higher rates out of larger than smaller stems which may be due to the lower C use efficiency of microbes (Manzoni *et al.*, 2008). Additionally, we found that N:P increased unequally across large and small stems as the decomposition process progressed. N:P ratios increased and stabilised at a value of 80 within large stems while staying unchanged and stabilising ~40 within small stems. The unequal dynamics in C:N and N:P ratios throughout the decomposition process between small and large stems suggest that microbial communities are exploiting their resources differently depending on the size of their resource. Microbial communities within wood include many specialists (McGuire & Treseder, 2010) and there are different species of fungi that target larger and smaller stems (Juutilainen *et al.*, 2011; Krah *et al.*, 2018). Such specialisation may have led to different stoichiometric needs and preferences in different sizes of wood.

Conclusions

We show that element accumulation and release patterns within deadwood have important similarities and differences with leaf litter, which could have consequences for ecosystem function including for N and P cycles in forests worldwide. Our novel results provide new insight into how microbes may be responding to the deadwood substrate by showcasing the stoichiometric balance and resource structural requirements for their optimal function. As C and N ecosystem models such as the MIcrobial-MIneral Carbon Stabilization model (Wieder *et al.*, 2014) or the Community Land Model (Lawrence *et al.*, 2019) increasingly incorporate details about N and C cycling within different substrates, moving beyond just leaf litter, our findings on the uniqueness of deadwood may be an early important step. If the results found here for 22 wood species hold across other taxa and systems, then the similarities and differences in wood C and nutrient release should be included. Future models could incorporate nutrient dynamics in relation to deadwood by assuming, for instance, that critical C:N at which N is released is ~110 and that decomposition stabilises at a C:N ratio of ~50. Additionally, nutrients are released at a mass remaining of ~40-100% depending on the nutrient considered and the initial concentrations of that nutrient within the wood. Finally, N:P ratios can increase during the decomposition process. These characteristics may emerge in conjunction with modern modelling frameworks if decomposers are also represented for their substrate preference e.g., leaf litter versus small stems versus large stems of deadwood. Such approaches would parallel the distinction in models among different plant growth forms

and reflect the growing body of knowledge on microbial functional ecology (Zanne *et al.*, 2020).

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Competing interests

We declare no competing interests.

Author contributions

AEZ, WKC, BO and JRP designed the experiment. WKC, JRP and BO performed the field data collection. BW performed the data analysis and interpreted the results with guidance from WKC and AEZ. BW wrote the manuscript with input from all the authors who have edited the draft.

Data availability

The data and code will be made available on a public repository.

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Supplemental information

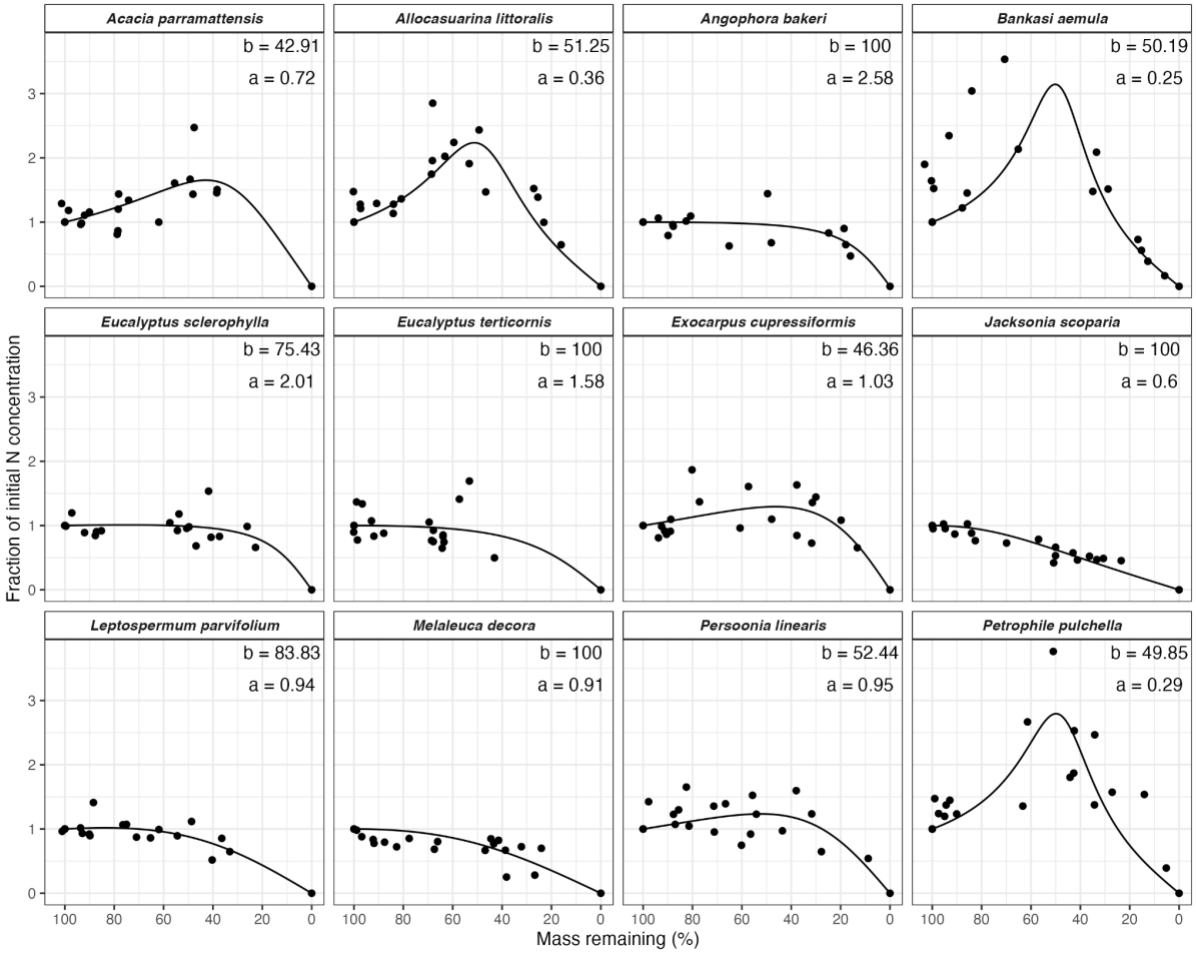

Figure S1 - Fraction of initial nitrogen concentration against mass remaining for each species within the larger stem category. The a and b values represent the outputs from the line of best fit for each species using equation 3.

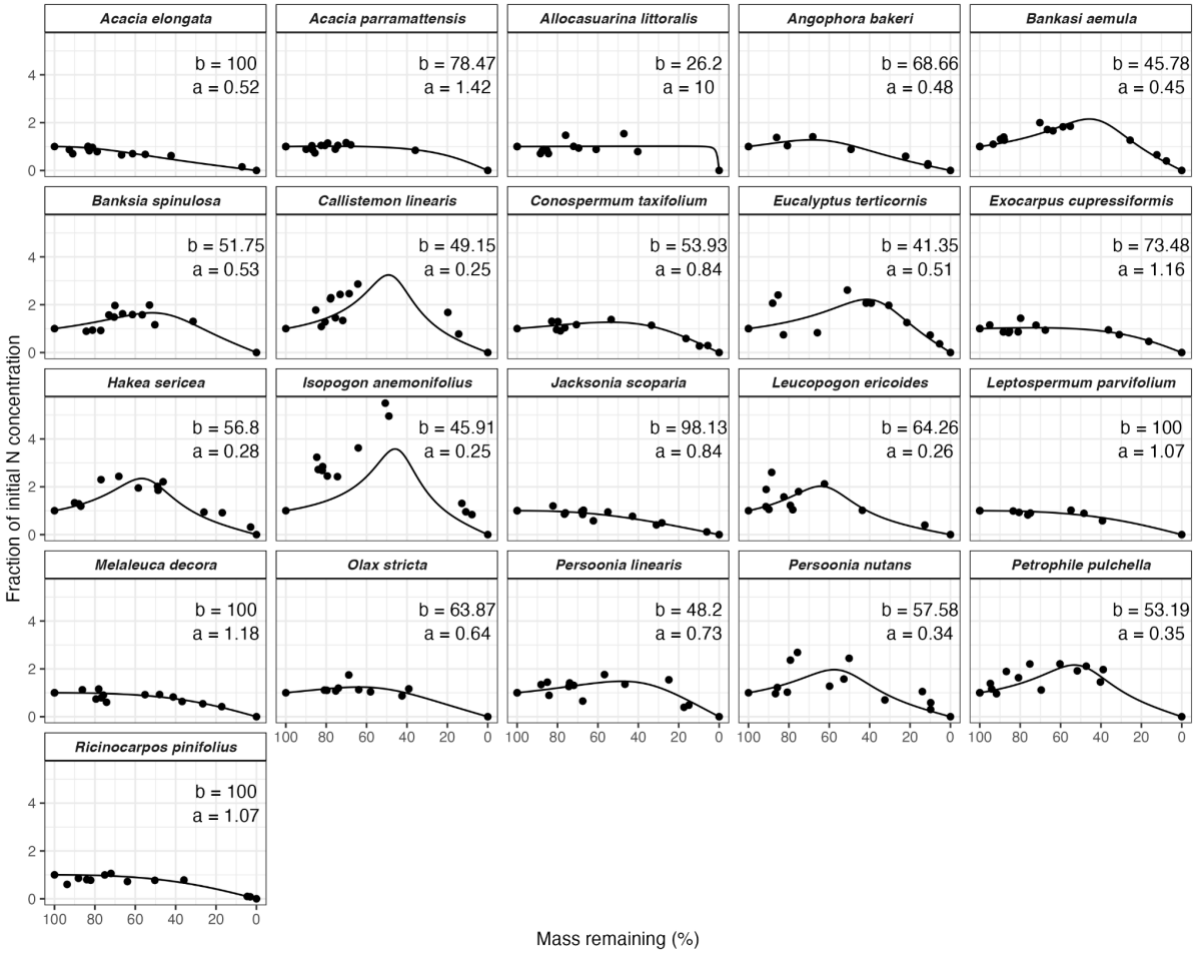

Figure S2 - Fraction of initial nitrogen concentrations for each species within the smaller stem category. The a and b values represent the outputs from the line of best fit for each species using equation 3.

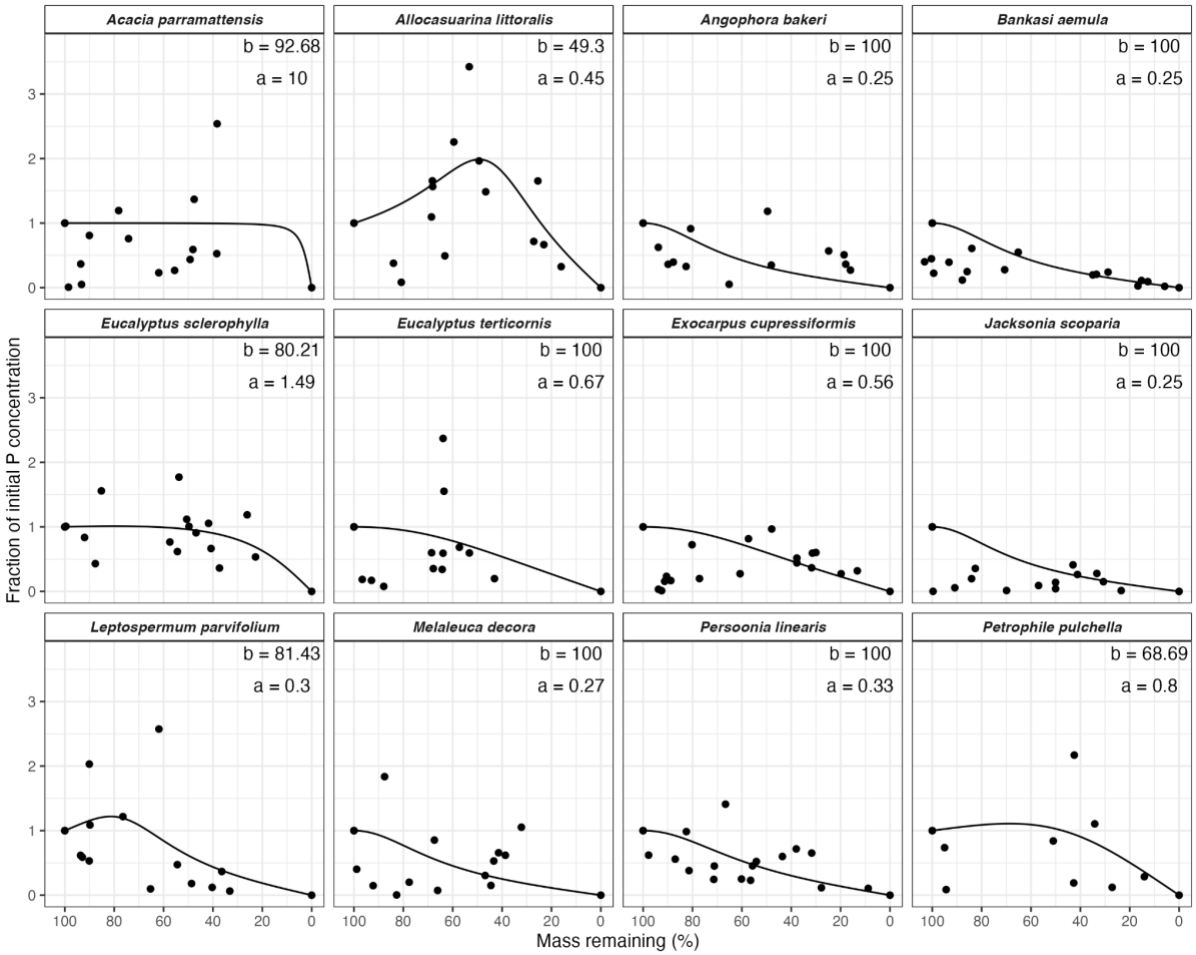

Figure S3 - Fraction of initial phosphorus concentration against mass remaining for each species within the larger stem category. The a and b values represent the outputs from the line of best fit for each species using equation 3.

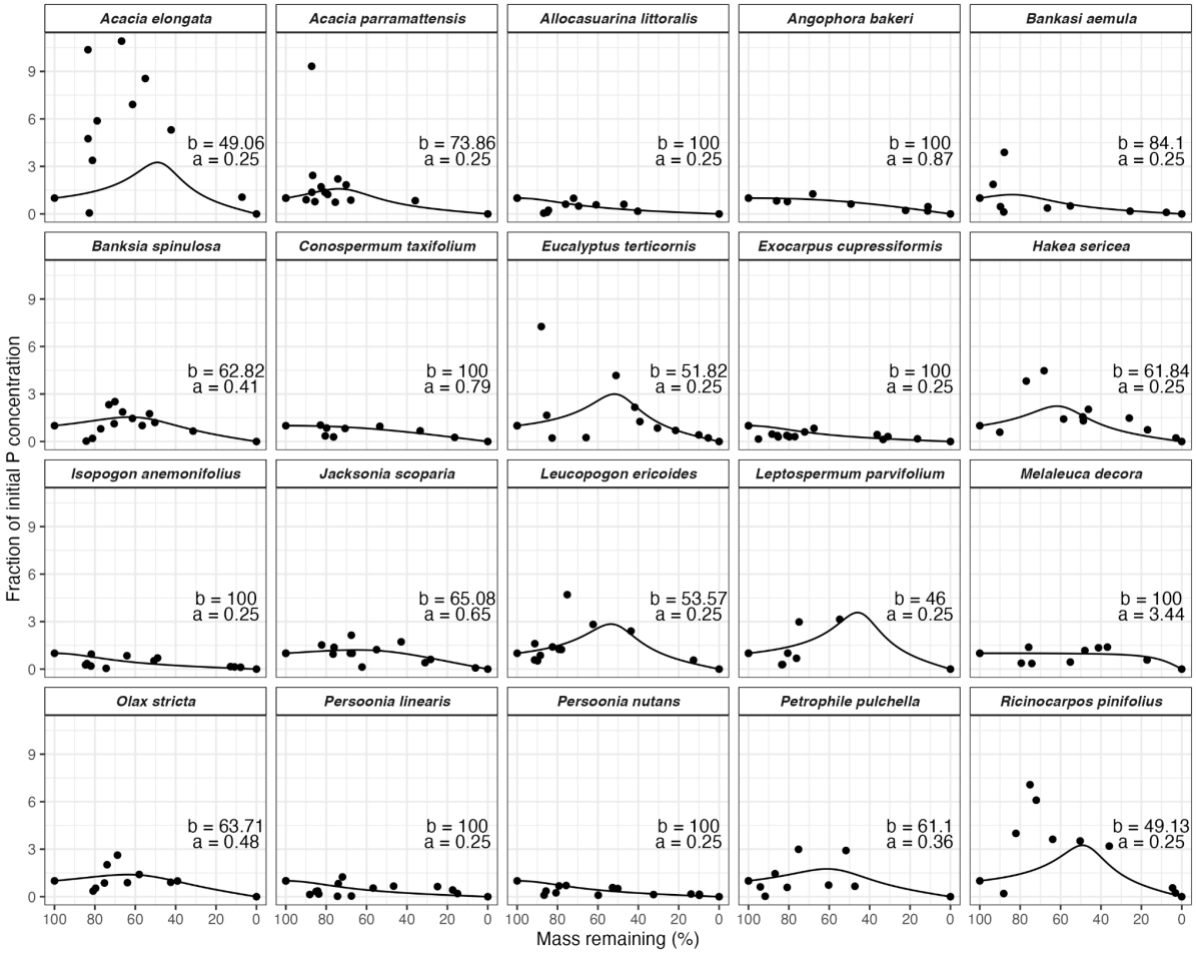

Figure S4 - Fraction of initial phosphorus concentrations for each species within the smaller stem category. The a and b values represent the outputs from the line of best fit for each species using equation 3.