# Stoichiometry of carbon, nitrogen, and phosphorus released from the leaf litter of various temperate tree species

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## Abstract

Dissolved organic matter and inorganic nutrients released from forest leaf litter through leaching are the important energy and nutrient sources that support the production of aquatic food webs. Litter leachate-derived dissolved organic carbon (DOC) is a critical energy source for aquatic heterotrophic microbes, and inorganic nitrogen and phosphorus can enhance primary production. In this study, we experimentally measured the release efficiencies and amounts of dissolved organic DOC, total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) of the leaf litter from 11 temperate tree species by soaking the leaf litter in water for 28 days. We found that the maximal release efficiency (% of element released per estimated mass of the element) was the highest for P and lowest for N. These efficiencies were species-specific. Additionally, the DOC:TDP and TDN:TDP ratios varied among the leachates of different leaf litter species and were considerably lower than the C:P and N:P ratios in leaf litter biomass; the DOC:TDN ratio was higher than the C:N ratio in leaf litter biomass. These results suggested that the ratios of organic carbon to nutrients dissolved into water cannot be fully elucidated using the elemental ratios of leaf litter mass. Based on these findings, we concluded that changes in the vegetations with different leaf litter stoichiometry can alter the relative importance of detrital and grazing food chains in aquatic ecosystems.

## Introduction

As in other ecosystems, the aquatic food web comprises detrital and grazing food chains (Lindeman 1942, Hairston and Hairston 1993). Terrigenous organic matters, such as leaf litter, are considered important energy and nutrient sources that sustain the food webs in ambient freshwater ecosystems. Organic carbon in the leaf litter can serve as a substrate for heterotrophic microbes in lakes and rivers (Carpenter et al. 2005, Cole et al. 2011, Hiltunen et al. 2017), thereby supporting the detrital food chains (Tanentzap et al. 2014, 2017, Hirama et al. 2022). When dissolved nitrogen and phosphorus are released from leaf litter and enter into aquatic ecosystems, they can be used for primary production by phytoplankton, which are the base of grazing food chains (Kissman et al. 2017, Hirama et al. 2022). Consequently, leaf litter can stimulate both the detrital and grazing food chains in aquatic ecosystems. However, the relative importance of the leaf litter in mass flow along the detrital and grazing chains may vary among the leaf litter species if the release rates of dissolved organic C (DOC), total dissolved N (TDN), and total dissolved P (TDP) differ among leaf litter species (Hirama et al. 2022).

The C, N, and P contents of leaf litter differ considerably among tree species (Schreeg et al. 2013, Yan et al. 2022). Furthermore, the release of nutrients from the leaf litter of coniferous species is lower and slower than from that of broadleaf species on soil (Usman 2013). Leaf litter from tree species with high lignin contents or high C to nutrient ratios generally decomposes more slowly and releases lower amounts of dissolved nutrients (Berg and McClaugherty 1989, Osono and Takeda 2004). Indeed, many studies have shown that the decomposition rate of the leaf litter has been shown to differ among tree species, suggesting that the release efficiencies of elements, i.e., fractions of DOC, TDN, and TDP in the C, N, and P contents of the leaf litter, respectively, differ among tree species.

P in leaf litter mainly exists as water-soluble orthophosphate and inositol phosphate (Chapin III et al. 1990, Yang et al. 2017). Therefore, the release efficiency of P is high and P is released rapidly during the early stages of litter leaching and decomposition (McComb et al. 2007, Schreeg et al. 2013, Pourhassan et al. 2016). In contrast, C and N in leaves exist mainly in structural components (Chapin III et al. 1990), such as lignin and cellulose, which are insoluble in water and difficult for most microbes to decompose (Johansson 1995, Kögel-Knabner 2002). Additionally, most of water soluble N in leaves are reabsorbed back to the trunk before defoliation in many species (Pate 1980). The fact suggests that the release efficiency of TDP from leaf litter is higher than that of DOC and TDN. However, few studies have examined the release efficiencies of these elements from the leaf litter soaked in water, which simulates the leaching and decomposition of leaf litter in freshwater ecosystems. Consequently, information regarding the tree species-specific release rates of DOC, TDN, and TDP of leaf litter in water is limited. If the release rates of these elements differ among the leaf litter of different tree species, it is likely that changes in the forest vegetation of a watershed can alter the relative importance of detrital and grazing food chains in aquatic ecosystems.

Therefore, in the present study, we investigated the C:N:P stoichiometry of leaf litter and the release efficiency of various tree species at the early stage of decomposition in a freshwater environment to examine whether changes in the watershed vegetation affect relative contributions of detrital and grazing food chains sustaining aquatic food webs. To this end, we soaked the leaf litter from 11 temperate tree species in aerated water and quantified the DOC, TDN, and TDP released over 28 days. Subsequently, we examined the following three uncertainties: does the release efficiency of leaf litter differ among DOC, TDN and TDP; are these release efficiencies species-specific; and what factors or elemental components in the leaf litter determine these release efficiencies?

## Material and methods

### *Collection and elemental content measurement of leaf litter*

We examined 11 tree species commonly found in the mountainous areas of northeast Japan. Among these species, we examined both young and aged leaf litter of oak (*Quercus serrata* ). Thus, we examined a total of 12 types of leaf litter. These shed leaves were collected at Zao (N38.122, E140.451) on October 3, 2020, at Kawatabi (N38.745, E140.757) on October 15, 2020, and at Aobayama (N38.259, E140.837) on November 19, 2020 in Miyagi Prefecture, Japan (Table 1). All leaves were collected within a week of falling on other leaf litter on the ground, and therefore did not directly come into direct contact with the soil.

The leaf litter was dried at room temperature (approximately 20 °C) in paper bags until it was used for measuring its C, N, and P contents. Prior to experiment, the C and N contents in the leaf litter were measured using an elemental analyzer (2400 Series II CHNS/O Analyzer, Perkin Elmer, Shelton, Connecticut). For estimating the P content in the leaf litter, the weighed amount of dry leaf litter was first combusted (420 °C) for 2 h to turn the litter into ash, which was suspended in 10 mL of distilled water. The mixture of ashes and distilled water was autoclaved with potassium persulfate for 30 min to oxidize organic phosphorus compounds and the total orthophosphate concentration was measured by ascorbate-reduced molybdenum blue method (Murphy and Riley 1962, Menzel and Corwin 1965).

### *Experiment*

Prior to the leaching incubation experiment, 1 L experimental bottles and ventilation equipment were washed with phosphorus-free detergent, and rinsed with 0.1 M HCl solution, and then rinsed with distilled water. We cut the dry leaf litter using scissors to mimic the initial decomposition process of detritivores and immersed the cut dried leaf litter into 1 L experimental bottles containing 800 mL distilled water. Two replicates of the leaching bottles were set up for each leaf litter species. These bottles were aerated and placed in dark incubators at 20 °C for 28 days. The dry weight of leaf litter used for leaching incubation was 7-385 mg, depending on the tree species: for tree species with low leaf litter P, a larger amount of leaf litter was added into experimental bottles to ensure that the P in leachate would be the same concentration level and thus minimizing and equalizing measurement errors.

During the experiment, we shook the bottles manually to mix the leachate once a day. On days 1, 3, 7, 14, 21, and 28, we sampled 50 mL of the supernatant of the leachate after all leaf litter had settled down to the bottom of the bottle. These samples were used to measure the DOC, TDN, and TDP in the leachate. DOC and TDN in the litter leachate were measured using a TOC/TNb analyzer (multi N/C 3100, Analytik Jena GmbH, Jena, Germany). For TDP measurements, 10 mL of leaf litter leachate was autoclaved with potassium persulfate and measured using the ascorbate-reduced molybdenum blue method.

*Estimation of release efficiencies of leaf litter C, N, and P*

We calculated the percentage of release relative to the total content of an element in the leaf litter as the release efficiency:

$V_{E,i} = \frac{D_{E,i}}{E}$ (1),

Where $D_{E,i}$ is the concentration of dissolved element $E$ (DOC, TDN, or TDP) at day $i$ and $E$ is the elemental concentration (C, N, or P, respectively) of the leaf litter added into the bottle. $E$ was calculated as follows:

$E = \frac{\text{Litter weight (mg)} \times \text{Litter } E \text{ mass } (\mu\text{g/mg})}{0.8(\text{liter})}$ (2).

We determined the maximal release efficiency ($V_{\max}$) and half saturation time ($k$) of an element $E$ by fitting the release efficiency $V_{E,i}$ and sampling day $t$ to the Michaelis-Menten equation as follows:

$V_{E,i} = V_{\max E} \frac{t}{k+t}$ (3).

The fitting was performed by non-linear least-squared regression using the *nls* function in the statistical package of R ver. 4.2.2 (R Core Team, 2022). The coefficient of determination ($r^2$) was estimated through correlation coefficient analysis between the observed $V_{E,i}$ and estimated $V_{E,i}$ from Equation 3.

We further calculated the maximal total release amount ($TRA$) of dissolved element $E$ for the 28-day experiment using $V_{\max E}$ and $E$ mass in leaf litter:

$\text{TRA}_E = V_{\max E} \times \text{Litter } E \text{ mass } (\mu\text{g/mg})$(4).

If $V_{\max E}$ from the Michaelis-Menten fitting was higher than 100%, we used the maximal release efficiency actually observed during the experiment (Max-$V_E$) instead for further analyses. To compare the C:P and N:P ratios of leaf litter and leachate, we also estimated the DOC:TDP, DOC:TDN and TDN:TDP ratios of $TRA$, i.e., $TRA_{\text{C:P}}$, $TRA_{\text{C:N}}$ and $TRA_{\text{N:P}}$. respectively.

*Statistical analysis*

We examined the differences in $V_{\max}$ between the leaf litter of tree species using pairwise Welch's $t$-test (Welch 1947) using the variance of these coefficients estimated with the *nls* function. In this test, alpha level for significance ($p < 0.05$) was adjusted using the Bonferroni correction. We examined the effects of leaf litter elemental contents and ratios on $V_{\max}$ (or Max-$V_E$) and $TRA$ using least-squared linear regression analyses.

## Results

*Elemental content of the leaf litter*

The C, N, P content and molar C:N, C:P, and N:P ratios varied considerably among the 12 types of leaf litter (Table 1). The C, N, and P masses ranged from 389.16 to 538.27 µgC/mg dry litter mass, 9.56 to 29.56 µgN/mg dry litter mass and 0.29 to 9.79 µgP/mg dry litter mass, respectively. The molar C:N ratio showed a 2.6-fold variation while the C:P and N:P ratios showed a 34-fold and 20-fold variations among the leaf litter, respectively.

*Release efficiency of DOC, TDN and TDP*

The release efficiency ($V_E$) against time was significantly fitted to the Michaelis-Menten equation ($p < 0.05$), as shown in the examples in Figure 1, except for TDN of aged oak (*Quercus serrata*) and Japanese larch

(*Larix leptolepis* ) leaf litter (Figure S1, S2, S3). The $V_{\max}$ estimated by fitting to the Michaelis-Menten equation exceeded 100% for TDP in leaf litter of Erman's birch (*Betula ermanii* ), young oak (*Quercus serrata* ), Japanese larch and hinoki cypress (*Chamaecyparis obtusa* ). The $V_{\max}$ of TDP was higher than those for of DOC and TDN in all the leaf litter examined. However, the half saturation time of release efficiency ($k$ ) was the shortest for DOC (2.41 days; Table S1), followed by TDN release (3.04 days; Table S1), and was the longest for TDP (16.99 days; Table S1). The $V_{\max}$for TOC, TDN, and TDP differed between the leaf litter types (Figure 2). Between broadleaf and coniferous leaf litter, the mean $V_{\max}$ of DOC (mean $V_{\max} = 22.54\%$ and 19.37%, respectively) and TDN (mean $V_{\max} = 5.20\%$ and 2.10%, respectively) of broadleaf species was higher ($t = 2.38$, $p < 0.01$ and $t = 7.44$, $p < 0.01$, respectively). However, no significant difference was detected in $V_{\max}$ of TDP between the broadleaf and coniferous species. We also examined the maximal release efficiency that was actually observed during the 28-day experiment (Max-$V_E$ ), which was, on average, 59.74 % for TDP across all species, and was also the highest among the elements, followed by that for DOC (20.76%), and the lowest for TDN (5.53%) (Table S1).

*Litter stoichiometry versus release stoichiometry*

No significant effects of the leaf litter content on $V_{\max}$ were detected for any of the elements (Figure S4a, e, i and Table S2). However, we found that $V_{\max}$ of DOC was positively associated with P content in the leaf litter (Figure 3a; Table S2).

We estimated $TRA_{\mathrm{C}}$, $TRA_{\mathrm{N}}$and $TRA_{\mathrm{P}}$, using $V_{\max}$ and the mass of these elements in the leaf litter (Figure S5). The $TRA_{\mathrm{C}}$ ($y = 7.82x + 78.29, r^2 = 0.347374859$, $p = 0.044$; Figure 3b) and $TRA_{\mathrm{P}}$ ($y = 0.41x + 0.32, r^2 = 0.79$, $p = 0.00011$; Figure 3c) were significantly and positively related to leaf litter P content but not to N and C contents. $TRA_{\mathrm{N}}$ was not related to any elemental content in the leaf litter.

Finally, we plotted molar C:P, C:N and N:P ratios of $TRA$ against those ratios of the leaf litter biomasses (Fig. 4). The results showed that both the $TRA_{\mathrm{C:P}}$ and $TRA_{\mathrm{N:P}}$ were considerably lower than the C:P and N:P ratios of leaf litter, while $TRA_{\mathrm{C:N}}$ was considerably higher than the C:N ratio of leaf litter. The $TRA_{\mathrm{C:P}}$ varied considerably (6.4 to 262.6) among the leaf litter and was higher in Japanese maple and zelkova (1 and 2 in Figure 4a). It was significantly and positively related to the C:P ratio of leaf litter biomass ($y = 0.065x - 15.12, r^2 = 0.77$, $p = 0.00011$; Figure 4a). The slope of the regression equation indicated that the release efficiency of P was, on average, 15 times higher than that of DOC compared with the elements in leaf litter biomass. Among the leaf litter, $TRA_{\mathrm{C:N}}$ ranged from 20.43 to 528.31 and was the highest in Japanese hemlock (8 in Figure 4b); $TRA_{\mathrm{N:P}}$ ranged from 0.05 to 3.24 and was higher in oak (aged) compared to the other leaf litter species (3 in Figure 4c). However, these ratios were not related to the C:N and N:P ratios in leaf litter biomass, respectively.

## Discussion

When the leaf litter of various tree species, including broadleaf and coniferous species, was soaked in water, we found that P had the highest release efficiency (>50 %), followed by C (20-30 %), and N release is the lowest (<10%). This was consistent with the results of previous studies examining the decomposition rate of the leaf litter of various tree species, showing that P was rapidly lost at the early stage of decomposition (McComb et al. 2007, Schreeg et al. 2013). The high release efficiency of P likely stems from the fact that P exists mainly as inorganic orthophosphate or inositol phosphate in plant tissues (Chapin III et al. 1990, Yang et al. 2017), which are highly water soluble and thus are leached from litter easily.

In this study, we successfully fit the time course changes in $V_E$ to the Michaelis-Menten equation, except for N in some species. However, the estimated $V_{\max}$ for P exceeded 100% in several leaf litter species including Erman's birch, oak (young), Japanese larch, and hinoki cypress (Table S1). In these species, the P release efficiency from the leaf litter increased almost linearly within our observation period (Figure. S3), indicating that 28 days was too short for TDP release to reach the saturation level of $V_{\mathrm{P}}$ in these species. Indeed, $k$ , the half saturation time, of P seemed to be considerably larger than that of C and N in all species. This trend suggests that the soluble parts of C and N in leaf litter were released more rapidly into the water than that of P, although the fractions of these parts in leaf litter were limited compared to that of P. In the leaf litter, although some of the organic C species, such as sugar, small molecular carbohydrates and organic acids are water soluble, most of the supporting structural carbon, such as lignin and cellulose, are water-insoluble (Kögel-Knabner 2002) and require more time to decompose (Nykvist 1963). Similarly, N biomass in leaf litter is mainly structural proteins. Although there are highly water-soluble nitrogen such as free amino acids, these are relatively minor in mass (Schneider et al. 1998, Franklin et al. 2020) and mostly recycled during leaf senescence before shedding (Chapin III et al. 1990, Nahm et al. 2006). Accordingly, regardless of species, the maximal release efficiency of P was considerably higher than those of C and N in the 28-day leaching experiment.

Studies based on water immersion of litter and litterbags suggest that the release efficiencies of DOC, TDN and TDP from deciduous broadleaf leaf litter are higher than those from evergreen coniferous leaf litter (Kiikkilä et al. 2011, Usman 2013, Pourhassan et al. 2016). Although we found higher DOC and TDN $V_{\mathrm{max}}$ in broadleaf leaf litter, we did not find a significant difference in the TDP $V_{\mathrm{max}}$ between the broadleaf and coniferous leaf litter because of the large variations within each of the two taxonomic groups. Interestingly, DOC, TDN, and TDP $V_{\mathrm{max}}$ (or Max-$V$ ) were not positively correlated with the C, N, and P contents, respectively, of the leaf litter (Figure S4a, e, i), suggesting that the soluble fraction of an element does not change proportionally with the elemental content in leaf litter. However, we found that DOC $V_{\mathrm{max}}$ increased with P content in the leaf litter (Figure 3a). These results are consistent with previous findings that more nutritious leaf litter contains more soluble organic C (Poorter and Bergkotte 1992). Although the TDP $V_{\mathrm{max}}$ was not related to the leaf litter P content itself, $TRA_{\mathrm{C}}$ and $TRA_{\mathrm{P}}$ were correlated positively with leaf litter P content. These results are consistent with those of previous studies showing that the leaf litter with relatively high P content generally has a high decomposition rate (Schlesinger and Hasey 1981, Osono and Takeda 2004). However, neither TDN $V_{\mathrm{max}}$ nor $TRA_{\mathrm{N}}$ were associated with the C, N, or P contents in the leaf litter mass and were consistently low relative to those of C and P. As mentioned above, these results may have been affected by the resorption of the water-soluble N in leaves by trees prior to shedding, regardless of the elemental contents in the leaves.

The C:P and N:P ratios of the maximal total release amounts in leachate, i.e., $TRA_{\mathrm{C:P}}$ and $TRA_{\mathrm{N:P}}$, varied considerably among the leaf litter and were substantially lower than the C:P and N:P ratios of the leaf litter biomass. In contrast, $TRA_{\mathrm{C:N}}$ was considerably higher than the C:N ratio for the leaf litter biomass. The result implies that the rates and ratios of organic carbon and nutrients released into water cannot be predicted solely through the elemental ratios of leaf litter mass. While $TRA_{\mathrm{C:P}}$ increased with increasing C:P ratio of leaf litter mass, no such increase was observed for $TRA_{\mathrm{C:N}}$ and $TRA_{\mathrm{N:P}}$. Furthermore, as $TRA$ is estimated from $V_{\mathrm{max}}$ and mass contents of leaf litter, and as $V_{\mathrm{max,\ P}}$ was not related to P contents, our results indicate that the P release efficiency is relatively less varied among the leaf litter species compared to the C and N release efficiencies. The variations of $TRA_{\mathrm{C:P}}$ with C:P ratio of leaf litter mass is thus associated with the P content and $TRA_{\mathrm{C}}$ but not necessarily with $V_{\mathrm{max,P}}$.

Traditionally, leaf litter is viewed as the substrate and nutrient sources for bacterial production in ambient aquatic systems (e.g., Cole et al., 2011; Lennon and Pfaff, 2005; Lindeman, 1942). However, the high P release efficiency supports the idea that leaf litter can also be a supplemental nutrient source for autotrophic organisms, such as algae, whose growth rates are often limited by P supply (Guildford and Hecky 2000, Sterner and Elser 2002). Among the leaf litter species, $TRA_{\mathrm{C:P}}$ was high in some species, such as Japanese maple and zelkova. Bacteria and algae compete for P in aquatic systems, and an increase in DOC supply often favors bacteria in the competition (Gurung et al. 1999, Thingstad et al. 2008, Hitchcock et al. 2010). Thus, an increased input of these leaf litter into ambient aquatic ecosystems may stimulate the heterotrophic microbial production more than the primary production in these ecosystems. However, $TRA_{\mathrm{C:P}}$ for the rest of leaf litter species, such as oak, Siebold's beech, Japanese larch and hinoki cypress, was considerably lower than 100, although the C:P ratios of these leaf litter mass were higher than 300 in most cases, and, on average, 660. Thus, these leaf litter species may stimulate the grazing food chains much more than the detrital chains by supporting the primary production in aquatic ecosystems. This possibility implies that the stoichiometric impact of leaf litter on aquatic ecosystems would be better understood if the water-soluble fractions of elements in the leaf litter species are considered.

Note that in this study, we incubated the leaf litter under a non-axenic condition, yet we used fresh distilled water to initiate the experiment. Thus, some fraction of the nutrients released from the leaf litter may have been fixed by microbes. However, if these bacteria were suspended in the experimental water, the fractions were included in the measured nutrients (TDN and TDP) in this study. The exception is some C fractions in leaf litter and DOC released may have been respired by microbes. This also implies that the $V_{\mathrm{C}}$ quantified in this study might be an underestimation. However, assuming this underestimation, it is likely that $V_{\mathrm{C}}$ would decrease with time. As such a trend was not observed, the effect of microbial respiration on $V_{\mathrm{C}}$, if present, would be minimal.

Finally, the stoichiometry of organic and inorganic nutrient release from leaf litter is likely to change depending on both leaf litter species and leaf litter senescence. Observations of oak leaf litter showed that the $V_{\mathrm{max}}$ of DOC and TDP were lower but that of TDN was higher in the aged leaf litter, which had lower N and P contents than young leaf litter (Figure 2). This trend may have been caused by C and P mineralization loss and N immobilization through microbial activity on leaf litter during the aging process on the soils (Gallardo and Merino 1992, Fellman et al. 2013). The immobilized N in aged leaf litter forms N-rich humus. A fraction of these compounds may have been released later following the immersion into water and decomposition of leaf litter (Gallardo and Merino 1992). To better understand these processes, it is necessary to examine how the senescence of leaf litter on soil affects the nutrient release efficiencies when it is soaked in water.

## Conclusion

In this study, we investigated the release efficiency of DOC, TDN and TDP of the 12 types of leaf litter from temperate forests. We confirmed that release efficiency was the highest in TDP during early leaf litter decomposition, followed by DOC, and TDN release efficiency was the lowest during the early leaf litter decomposition, regardless of the species. More importantly, we found that the maximal release efficiencies and amount of DOC, TDN and TDP from leaf litter were highly species-specific. Furthermore, we found that the C:P and N:P ratios of the total maximal release amounts were considerably lower than the C:P and N:P ratios in leaf litter biomass, while the C:N ratio of total maximal release amount was higher than the C:N ratio in leaf litter mass. Considering the fact that leaf litter enters ambient aquatic ecosystems, the present results suggest that changes in the vegetations due to factors such as plantations and climate change can alter the relative importance of aquatic detrital and grazing food chains (Prentice et al. 1991, Hirama et al. 2022).

## Significance statement

Dissolved organic matters and inorganic nutrients released from forest leaf litter are important energy and nutrient sources that support the production of aquatic food webs. However, the variation of the release efficiencies of dissolved organic matter and inorganic nutrients of leaf litter with different stoichiometric traits and from distinct tree types are elusive. Our study revealed that release efficiency of dissolved C, N, and P is highly species-specific and partially related to leaf litter stoichiometry. This inferred that changes of the vegetation with different leaf litter stoichiometry in watersheds can alter the food web structure in the ambient aquatic ecosystems. We believe that these results, notably on the stoichiometry of forest leaf litter and leaf litter leachate and its cross-ecosystem effects on aquatic food webs have a broad appeal among both aquatic and terrestrial ecologists.

## Data archiving statement

We will deposit our data and codes for analysis on Dryad.

## Conflict of interest statement

The authors declare no conflict of interests.

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| **Tree species** | **Tree species** | **Tree species** | **Tree species** | **Sampling site** | **Sampling site** |
|---|---|---|---|---|---|
| Common name | Species name | Tree type | Leaf type | Area | Latitude |
| Japanese maple | *Acer amoenum* var.amoenum | Broadleaf | Deciduous | Aobayama | N38.259 |
| Zelkova | *Zelkova serrata* | Broadleaf | Deciduous | Aobayama | N38.259 |

| Tree species | Tree species | Tree species | Tree species | Sampling site | Sampling site |
|---|---|---|---|---|---|
| Erman's birch | *Betula ermanii* | Broadleaf | Deciduous | Zao | N38.129 |
| Oak (brown) | *Quercus serrata* | Broadleaf | Deciduous | Zao | N38.122 |
| Oak (green) | *Quercus serrata* | Broadleaf | Deciduous | Kawatabi | N38.745 |
| Siebold's beech | Fagus crenata | Broadleaf | Deciduous | Kawatabi | N38.745 |
| Japanese elm | *Ulmus davidiana* var. japonica | Broadleaf | Deciduous | Kawatabi | N38.745 |
| Japanese hemlock | *Tsuga diversifolia* | Coniferous | Evergreen | Zao | N38.122 |
| Japanese cedar | *Cryptomeria japonica* | Coniferous | Evergreen | Kawatabi | N38.745 |
| Japanese red pine | *Pinus densiflora* | Coniferous | Evergreen | Kawatabi | N38.745 |
| Japanese larch | *Larix leptolepis* | Coniferous | Deciduous | Kawatabi | N38.745 |
| Hinoki cypress | *Chamaecyparis obtusa* | Coniferous | Evergreen | Kawatabi | N38.745 |

**Table 1.** Stoichiometry of leaf litter collected in Miyagi prefecture, Japan. The standard deviation of the elemental contents with multiple measurements are provided in the parentheses.

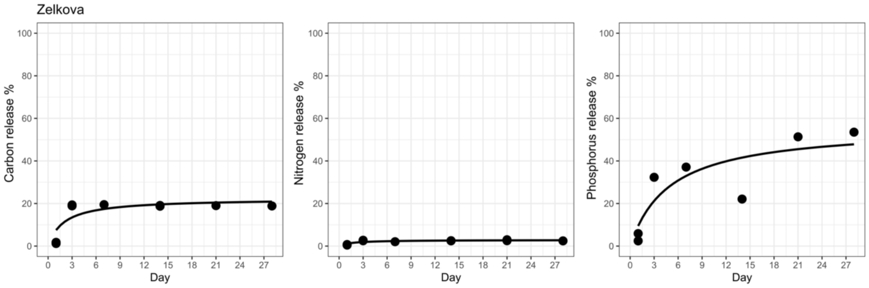

**Figure 1.** Examples of the Michaelis-Menten equation fit to the percentage of C, N, and P release ( $V_E$ ) during the 28-day of incubation experiment.

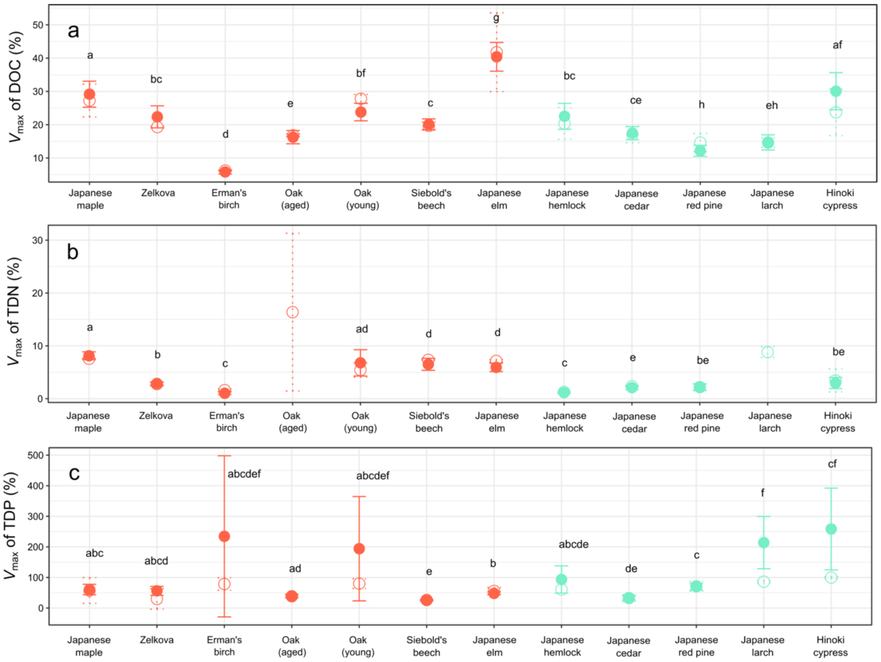

**Figure 2.** Maximal release efficiency ($V_{\mathrm{max}}$) and its standard deviation for dissolved organic carbon (DOC) (a), total dissolved nitrogen (TDN) (b), and total dissolved phosphorus (TDP) (c) estimated from fitting the Michaelis-Menten equation (solid circles and lines) and from the observed maximal release (Max-$V_E$) during the 28-day leaching experiment (hollow circles and dotted lines). Red circles denote broadleaf and aqua blue circles denote coniferous leaf litter. Significant differences of $V_{\mathrm{max}}$ estimated from the Michaelis-Menten equation fitting are labeled with different letters.

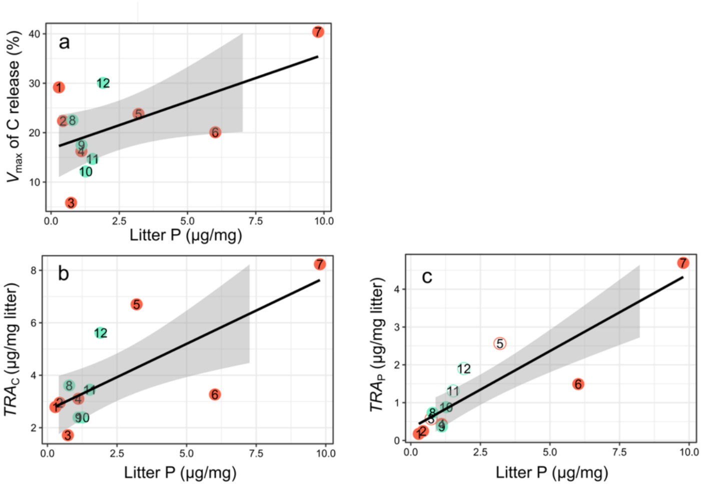

**Figure 3.** Linear regression between (a) maximal release efficiency ($V_{\mathrm{max}}$) of dissolved organic carbon (DOC), (b) maximal total release amounts of DOC ($TRA_{\mathrm{C}}$) and (c) total dissolved phosphorus (TDP) per

mg leaf litter dry mass ( $TRA_{\mathrm{P}}$) against P contents in leaf litter. Open circles in subplots denote observed maximal release efficiency (Max- $V_E$ ) from the incubation experiments that are estimated for tree species with $V_{\mathrm{max}} > 100\%$ (P release from Erman's birch, oak (young), Japanese larch and hinoki cypress). Numbers signify tree species in the order outlined in Figure 2. Significant linear regression and the estimated 95% confidence interval of the linear fitting are presented as solid lines and shaded areas.

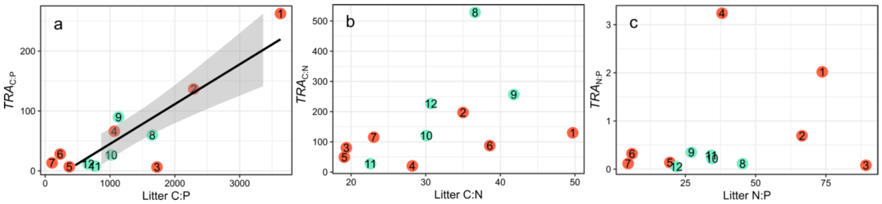

**Figure 4.** Linear regression between (a) molar C:P ratio of maximal total release amount ( $TRA_{C\ :\mathrm{P}}$) and the C:P ratio of leaf litter biomass, (b) molar C:N ratio of maximal total amount release ( $TRA_{C\ :\mathrm{N}}$) and the C:N ratio of leaf litter biomass and (c) molar N:P ratio of maximal total amount release ( $TRA_{N\ :\mathrm{P}}$) and the N:P ratio of leaf litter biomass. Red circles denote broadleaf leaf litter and aqua blue circles denote coniferous leaf litters. Numbers signify tree species in the order outlined in Figure 2. Significant linear regression and the 95% confidence interval of the linear fitting are presented as solid lines and shaded areas.