Leaf litter decomposition in boreal lakes: variable mass loss and nutrient release ratios across a geographic gradient

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Abstract

Here, we assess regional differences in decomposition rates of allochthonous plant detritus in the littoral zones of lake ecosystems. Specifically, we measured breakdown rates and elemental composition of aspen leaves (*Populus tremuloides*) over 60-70 days in 14 lakes from four lake regions located >1,000 km apart in Ontario, Canada. We found substantial differences in leaf breakdown among regions with much faster rates seen in more nutrient-rich and warmer lakes. While breakdown rates increased slightly with larger mesh size, which provided greater access by macroinvertebrates, these effects were negligible compared to those produced by regional differences in nutrients and temperature. We also found regional differences in detrital nutrient release, with variable N- or P-specific fluxes and their ratios, which indicates differential release of these nutrients back into the lake's water column. Release ratios varied most in litterbags that showed the least mass loss, which indicates microbial uptake and release dynamics of N and P can uncouple under low nutrient conditions. Our results demonstrate that terrestrial leaf material and its associated nutrients may experience contrasting fates among lakes in the boreal landscapes with possible effects on lake nutrient cycles.

Keywords: macroinvertebrates, ecological stoichiometry, breakdown rates, nutrient ratios, carbon dynamics, lakes

Introduction

Leaf litter is an important external resource subsidy to many freshwater ecosystems. As this material flux can represent an impressive amount of organic matter into streams (Fisher & Likens, 1973, Gulis & Suberkropp, 2003a), the decomposition of terrestrial leaf inputs and its environmental controls has been a central research area for lotic ecologists for decades (Webster & Benfield, 1986; Graça et al., 2005). Although considerable quantities of leaf litter can be added to lake surface waters each fall (Pope et al., 1999) and terrestrially derived organic matter are important materials supporting lake food webs (Cole et al., 2006), the decomposition of terrestrial leaf material has not received the same degree of study in lakes as it has in streams and rivers. Consequently, comparatively little is known about rates of leaf litter decomposition, its variability, or its effects on nutrient cycles in temperate lakes.

Decomposition rates of leaf material could vary among lakes due to differences in the local environment. Higher rates of mass loss are often reported for nutrient-rich ecosystems (Gulis & Suberkropp, 2002; Leroy & Marks, 2006; Greenwood et al., 2007), as nutrient subsidies in the water column appear to accelerate decomposition by supplementing low nitrogen (N) and phosphorus (P) supplies to microorganisms provided by carbon (C)-rich, mineral-poor leaf substrates (Gulis & Suberkropp, 2003a; Greenwood et al., 2007). Higher nutrient concentrations increase microbial growth and accelerate their breakdown of leaf material. Leaf decomposition also varies due to differences in water temperature (Swan & Palmer, 2004; Ferreira et al., 2006; Ferreira & Canhoto, 2014). Breakdown rates in lakes and streams generally increase with water temperature due to higher metabolic activity of microorganisms (Carpenter & Adams, 1979; Chauvet & Suberkropp, 1998). Slower leaf breakdown rates due to low water temperature have been documented in stream ecosystems across elevation, latitudinal, and

61 geothermal gradients (Webster & Benfield, 1986). These results provide a strong rationale for
62 predicting that leaf breakdown rates in lakes will increase with greater nutrient concentrations
63 and ambient water temperature.
64 Decomposition rates are also affected by the presence and abundance of
65 macroinvertebrates (Graça, 2001; Leroy & Marks, 2006). While macroinvertebrates can
66 accelerate leaf decomposition (Cummins et al., 1973; Wallace et al., 1982; Hieber & Gessner,
67 2002), this effect appears to be highly context dependent (Brown & Ricker, 1982; Short et al.,
68 1984). This context dependency reflects the species composition of macroinvertebrate
69 communities, specifically the relative abundance of shredding detritivore taxa (Graça, 2001;
70 Hieber & Gessner, 2002). Because of the low N and P content within leaf material, shredder
71 nutrition appears to be largely derived from nutrient rich leaf microbial biofilms (Cummins et al.
72 1973, Cross et al., 2003). Nutrient enrichment of these microbial communities may reduce
73 shredder-resource elemental imbalances and lead to higher shredder biomass and feeding rates
74 (Cross et al. 2003, Greenwood et al. 2007). While shredder effects can be substantial in riverine
75 ecosystems where detritivore activity is often a predominant controller of leaf litter breakdown
76 rates, less is known about how macroinvertebrates affect leaf breakdown in lakes.
77 Macroinvertebrates have been found to increase breakdown rates of leaves in some lakes
78 (Tuchman, 1993; Sabetta et al., 2000) and have no effect in others (Raposeiro et al. 2017). It may
79 be that site-specific and regional factors such as nutrient supplies or water temperature control
80 the relative influence of microbial versus detritivore mediated leaf breakdown in lakes.
81 Variable rates of leaf breakdown should affect the rates and ratios of nutrients fluxing in
82 and out of detritus. During breakdown, nutrients are released as organic material is degraded by
83 colonizing detritivores. Complete organic decomposition (i.e., 100% mass loss) must result in

84 nutrients being released in ratios proportional to that initially present in deposited detrital
85 material (Banks & Frost, 2017). Deviations from this first-order prediction can occur during
86 microbial-mediated decomposition when organic breakdown and losses becomes uncoupled
87 from microbial acquisition and release of mineral nutrients. For example, ratios of released
88 nutrients might vary if there is sustained carbon loss during breakdown but immobilization of
89 nutrients by microorganisms through colonization and biomass accrual (Mehring et al., 2015)
90 due to the high C and low nutrient content of plant detrital matter. As most decomposition
91 studies primarily focus on mass loss and nutrient sequestration (Gessner et al., 1999), less is
92 known about variation in nutrient fluxes from decomposing leaf material, especially in lakes that
93 vary in nutrient concentrations and water temperature.
94 Here, we assessed leaf breakdown and nutrient fluxes in lakes along a latitudinal gradient
95 (~1200 km) across Ontario, Canada. This spatial gradient was accompanied by a range of lake N
96 and P concentrations and water column temperatures, which allowed us to examine relationships
97 between decomposition and these environmental factors. While measuring decomposition rates
98 (k, day^{-1}) of trembling aspen leaves (*Populus tremuloides*), we also assessed the possible role of
99 macroinvertebrates through the use of different mesh sizes in our decomposition bags. In
100 addition, we measured the nutrient content of the leaf detritus before and after the experiment to
101 examine how ratios in released nutrients were affected during decomposition. We predicted that:
102 i) breakdown rates of leaf litter would vary among lake regions due to differences in lake nutrient
103 concentrations and water temperature, ii) larger mesh sizes would allow more macroinvertebrate
104 colonization and increase leaf litter breakdown rates but that this effect could vary with lake
105 temperature and nutrient concentrations, and iii) nutrient ratios in released materials would vary

among regions and across this environmental gradient but the net effect would depend on lake temperature and nutrients.

Methods

Study sites. We conducted this study in 14 lakes within four distinct regions in Ontario, Canada (Figure 1) that cover a range in physiochemical conditions. The Kawartha Lakes region is found south of the Canadian Shield and is characterized by highly developed shorelines and significant agricultural land use (Crins et al., 2009; Banks & Frost, 2017). We selected four lakes (Stoney, Sturgeon, Chemong and Pigeon Lake) in this region, which is located near Peterborough, Ontario. During our study, total phosphorus (TP) concentrations in these lakes ranged from 25-33 µg L^{-1} (Table 1). We also included three lakes from the Kawartha Highlands Provincial Park (Coon, Long, and Mississauga), which lies on the southern boundary of the Canadian Shield and is a forested, undeveloped landscape with the exception of some recreational shoreline development (Frost & Hicks, 2012). Lakes within this region are generally relatively P-poor (Schindler et al., 1978), we found concentrations of TP ranged from 18-33 µg L^{-1} during our study (Table 1). Three of our lakes (Heeney, Blue Chalk and Harp) were located near Dorset, also in south-central Ontario. These lakes are typically lower nutrient, mesotrophic lakes (Keller et al, 2008). Concentrations of TP in these lakes ranged from 19-21 µg L^{-1} during our study (Table 1). We also studied four lakes (L224, L239, L373, and L626) at IISD-Experimental Lakes Area (IISD-ELA) in northwestern Ontario. This region is located entirely on the Canadian Shield and has a landscape characterized by granite bedrock and coniferous forests. IISD-ELA lakes are relatively undisturbed by human activities and are oligotrophic (Schindler et

al., 1973). During our study, TP concentrations in these lakes ranged from 10-12 µg L^{-1} (Table 1).

Leaf litter collection and experimental design. We collected senesced aspen leaves (*P. tremuloides*) in April 2016 from the Trent University campus located in Peterborough, Ontario. On collection, leaves showed no sign of decomposition, were free from soil and other debris, and did not require rinsing. Aspen was chosen because it is a common species found across the geographic gradient of this study. We dried leaves at room temperature (19-22°C) for approximately two weeks before leaf bag construction. For each lake, we prepared four replicate bags for each of two separate mesh sizes, which yielded a total of 112 litterbags. We placed 15 grams (+0.05 g) of aspen litter, marginally more than recommended by Hauer and Lamberti (2006), in each leaf litter bag (5 cm x 20 cm) of both mesh sizes. Small mesh (300 µm) was used to exclude invertebrates whereas a larger mesh (1 cm) was used for bags that were open to invertebrate colonization. Litterbags were anchored to the lake bottom using 1 meter of rope with cinderblocks attached to each end. Litterbags were placed into the littoral zones in each lake on dates between May 31st and June 10th, 2016. Bags were placed at 1.5 meter depth to ensure that they would remain submerged over the course of the experiment. After allowing approximately 60 days of incubation in lakes, bags were removed from lakes on dates between July 27th and August 17th, 2016. Bags were removed carefully from each location and no leaf loss was seen during removal of bags. Following removal from each site, bags were kept in separate plastic bags, held on ice, returned to the lab, and processed within 24 h of retrieval. In the laboratory, leaf litter was rinsed with deionized water over a 250 µm mesh sieve to remove sediment and invertebrates from large mesh litter bags. Invertebrates, even smaller ones (e.g., Chironomidae), were not observed in any of the small mesh litter bags. Litter was air dried at room temperature

(19-22°C) until reaching constant mass. After weighing, we ground samples from each of the four replicate litter bags for subsequent elemental analyses.

Nutrient and lake water chemistry processing and analyses. Temperature was recorded hourly in each lake for the entire duration of the experimental incubation using data loggers (Onset HOBO). Water samples were collected at each site during the initial placement of bags in lakes, after approximately 30 days and, when bags were removed, for analyses of phosphorus (TP), nitrogen (TDN), pH, and dissolved organic carbon (DOC; Table 1). In the laboratory, we filtered and saved analytical (n=2) samples for one aggregate water sample within 2-6 hours of collection. We preserved whole water samples for TP and filtered remaining samples through 0.2 μm polycarbonate filters. These samples were stored for no more than 4 weeks in the dark at 4°C until analyses were run.

We measured the elemental composition of leaf litter saved at the start of the experiment and following leaf bag removal using homogenized with mortar and pestle, composite subsamples from each of the four replicate bags collected from each lake. Samples (2-4 mg dry mass) of this powder were used for analysis of detrital carbon (C) and nitrogen (N) content using a CN analyzer (Vario EL III, Elementar). A separate subsample was analyzed for P content after ashing at 550°C for four hours. TP samples were measured by persulfate digestion using a molybdate-blue colorometric method (APHA, 1992) using a spectrophotometer (Cary-50, Varian). We analyzed TDN samples by oxidation with sodium hydroxide-persulfate and determined concentrations using a second derivative spectroscopy method (Crumpton et al., 1992).

Data analysis. We calculated decomposition rates (k; day^{-1}) using the slope from the regression of the natural log-transformed litter mass over time (Petersen & Cummins, 1974).

While our design used only two data points to estimate this slope, we calculated decomposition rates the same way for all treatments and lakes using this equation:

$$k\ (day^{-1}) = \frac{\ln(initial\ mass) - \ln(final\ mass)}{time\ (day)}$$

We thus recognize the inherent uncertainty in our *k* estimates but were limited to two measurements due to logistical constraints created by our broad spatial study design. Element-specific breakdown rates (fluxes) were calculated as above but with the total litterbag content of C, N, and P used in place of mass. The change in nutrient content of each litterbag was calculated by subtracting the total nutrient content of each bag at the end of the experiment from the estimated initial value.

We used a mixed-effect general linear model to determine the effects of region, mesh size, region*mesh interaction, and random lake effects nested within each region on decomposition rate (Sokal & Rohlf 1997). We visually examined residual plots after fitting the model and found one data point from Pigeon Lake that caused our dataset to violate normality and homogeneous variance assumptions of parametric statistics. Therefore, we removed this point, reran the model, and excluded this point from further statistical analyses (Gotelli & Ellison 2004). Significant model effects were determined using Bonferroni adjusted F-ratio tests ($p=0.05/4=0.0125$; Sokal & Rohlf 1997). Effect sizes for each variable were calculated as eta-squared values. Eta-squared values are useful for comparing the relative influence of environmental variables on a given response variable (i.e., decomposition rates) and are calculated by dividing the sum of squares for each model term by the total model sum of squares (Levine & Hullett 2002; Richardson, 2011). To compare regional differences in decomposition rates, we conducted multiple comparisons using least squared means t-tests. Next, we used both partial least squares (PLS) regressions and ordinary least squares (OLS) regression to examine

197 relationships between environmental factors and decomposition rates. As *k* values represent
198 integrated measurements across the entire study, we regressed *k* values against mean
199 temperature, TP, TDN, and pH values averaged across 3 sampling dates. As TDN concentrations
200 from IISD-ELA fell below detection limit, we used a mid-point value (25 µg L^{-1}) between 0 and
201 the detection limit of 50 µg L^{-1} for this analysis. We assessed regional differences in C:N, C:P,
202 and N:P release ratios (all molar) by regressing loss (Initial C- Final C) of numerator elements
203 against the denominator and comparing the slopes of regressions across regions using *t*-tests,
204 which were Bonferroni corrected for multiple comparisons. All statistical analyses were
205 completed on SAS (University Edition).
206
207 **Results**
208 *Environmental controls of leaf decomposition rates.* Across regions, there was
209 considerable variability in litter breakdown rates (Figure 2), which corresponded to a wide range
210 in total mass loss (3-88% of original mass) over the 60-day incubation. Breakdown rates were
211 slowest at the IISD-ELA, moderately higher in the Dorset and Highlands Lakes, and fastest in
212 the Kawartha Lakes region (Figure 2). A majority of the variability among lake decomposition
213 rates was attributable to region (r^2=0.44), but we also found substantial variation in
214 decomposition rates among lakes within each region (r^2=0.39), especially in the Kawartha Lakes
215 (Table 2; Figure 2). Mesh size effects on leaf breakdown were insignificant (p=0.023; Table 2)
216 and there were no region*mesh size interactions (p=0.082), which indicates that invertebrate
217 feeding effects on decomposition rates were minimal across the study region.
218 The breakdown rates of aspen leaves were not correlated with the DOC concentrations in
219 lakes of our study (p>0.05) but were significantly related to all other environmental factors that

we measured (Figure 3). There was high collinearity among all of these variables, which could not be eliminated (i.e., setting them orthogonal to one another in Euclidian space) using more complex PLS-regressions. As the relative influence of each variable on decomposition rates was virtually identical between the two regression analyses, we report the results of OLS regressions, which allow us to also highlight regional differences in environmental variables. Decomposition rates increased with increasing temperature, pH, TP, and TDN concentrations (Figure 3). These relationships reflect a regional gradient in decomposition with slow breakdown in low nutrient, cold lakes in the north and faster breakdown in high nutrient, warm lakes in the south (Figure 3). Of these variables, lake TP and TDN were most strongly related to leaf litter breakdown rates followed by pH and temperature.

Element-specific flux and nutrient release ratios during decomposition. At the start of the experiment, the elemental composition of *P. tremuloides* was %C= 48.2, %N= 1.13, and %P= 0.09 (molar ratios of C:N= 49.5, C:P= 1344, and N:P= 27.3). The rate that detritus lost these elements (i.e., element-specific breakdown rates) was tightly coupled to the mass-specific rate of leaf litter breakdown. C-specific decomposition rates were nearly identical to mass-specific decomposition rates (slope = 0.97, $r^2 = 0.99$, $p<0.001$), whereas N- and P-specific breakdown rates were less tightly coupled to rates of mass decomposition (slope =1.09, $r^2 = 0.95$, $p<0.001$ and slope = 0.95, $r^2 = 0.90$, $p<0.001$, respectively). These relatively weaker correlations for N- and P-specific breakdown were mostly attributable to high variation in N and P net release at lower mass-specific decomposition rates (those with $k<0.01$ day^{-1}). Corresponding to this, there was high variability among the ratio of N- and P-specific decomposition (i.e., k_N/k_P) when most of the litter mass remained at the end of the experiment, whereas the ratio of these element-

specific loss rates showed more consistent values when most of the litter mass was lost over the duration of the experiment (Figure 4).

Differences in stoichiometric release ratios reflected the balance of uptake and release of both N and P. There was a net release of N from nearly all bags, but it was released at proportionally slower rates than C. This yielded relatively high C:N ratios (>50) in released materials from bags incubated all four study regions (Figure 5). Release ratios for C:P varied considerably with some values falling above and some below the C:P ratio of the initial litter. These differences, in part, were accounted for by region. At the IISD-ELA, released C:P ratios were very high (≥2500) and well above those measured in initial leaf litter. Release ratios from litterbags incubated in lakes of Dorset and the Kawartha Highlands varied considerably with values above and below the initial C:P line (1350) but, on average, they did not differ significantly from the IISD-ELA (Table 3; $p>0.4$). In contrast, C:P release ratios in the Kawartha Lakes region differed from all 3 sites ($p<0.02$) and were most similar to initial values. These differences in released C:N and C:P ratios were accompanied by variable N:P release ratios among regions ($p<0.01$). At the IISD-ELA, released N:P ratios were observed both above and below the initial N:P ratio line (Figure 5). Release ratios of N:P were more variable in the Dorset and Kawartha Highlands lakes, but they were generally lower than the initial litter N:P ratios due the effects of relatively lower N and higher P release. Ratios of N:P released during litter decomposition were most variable among the Kawartha Lakes. In this region, P release was greater than N in locations having low breakdown rate (values below the initial N:P), but in lakes with faster decomposition, N:P release ratios approached those of initial litter (approaching the line denoting the initial N:P ratios; Figure 5). While we found regional differences in release

ratios of C:N, C:P, and N:P from the detritus, there were no significant relationships between these variables and lake TP or temperature (data not shown).

Discussion

Rates of decomposition varied among our four study regions and were related to differences in water temperature and nutrients. Variable breakdown rates within and among these regions produced differential fluxes of C, N, and P back into the lake water column. Our results show that litter breakdown rates can vary substantially even among relatively similar lakes in what otherwise appears to be a relatively homogeneous, boreal landscape. Similarly, we found variable nutrient ratios released from decomposing detritus, which could differentially affect water column nutrient dynamics. Our study shows that more work is needed to study the fate of terrestrial detritus in lake ecosystems, especially given expected changes in temperature and nutrient concentrations in temperate lakes in the coming decades (Ferreira & Chauvet, 2011a).

Decomposition rates varied widely across our study lakes and among the lake regions in our study. Much of the variability in decomposition rates was a result of rapid leaf breakdown in the southern Kawartha Lakes region compared to the slower breakdown rates at the more northern sites of the IISD-ELA region. We found these regional differences in decomposition rates to be most strongly correlated with higher lake water N and P concentrations. Our results likely reflect the enhanced growth and activity of decomposer microorganisms that are stimulated with nutrient enrichment (Gulis & Suberkropp, 2002), which can alleviate C-nutrient imbalances between microbes and detrital resources (Frost et al., 2002; Cross et al., 2003). Reduced stoichiometric constraints increase growth rates and gross growth efficiencies of C by relaxing mineral nutrient limitation in microbial decomposers (Gulis & Suberkropp, 2003b;

Suberkropp et al., 2009). Altogether, these effects of nutrients on microbial colonizers should result in increased rates of detrital breakdown, which is consistent with our findings here.

We also found that leaf litter decomposed faster in warmer lakes. This is consistent with well-established temperature sensitivity of microbial activity with faster metabolism and growth of these organisms in warmer water (Ferreira & Chauvet, 2011b; Martínez et al., 2014, Tiegs et al. 2018). However, despite this positive relationship with water temperature, we measured a wide range of breakdown rates among lakes in our warmer southern study regions (Dorset, Kawartha Highlands, and Kawartha Lakes). High variability among these lakes within a relatively narrow temperature range (<2°C) indicates that other environmental constraints, most likely nutrient limitation, interact with temperature to control leaf litter breakdown in these lakes. While our data is not well-suited to fully assess interactive effects of nutrients and water temperature, our results are generally consistent with expectations based on this concept (Philips et al., 2017). In addition, we found significant correlations with lake pH, which we suspect reflects co-variation between this variable with temperature and nutrient concentrations. Nonetheless, our findings demonstrate that regional variation in lake temperature and nutrients together can produce large differences in litter decomposition rates and, by extension, C storage in lakes embedded in the boreal landscape. Future work is needed to examine how decomposition rates relate to variable temperatures at fixed nutrient concentrations and vice versa in these lakes (Philips et al., 2017) to better understand the strength and nature of interactive effects between these two environmental variables.

We found that leaf detritus decomposition was not noticeably affected by macroinvertebrates in our study lakes. This is consistent with the small percentage (~6%) of experiment-wide variability in decomposition rates accounted for by bag mesh size, which was

far less than that accounted for by temperature and nutrients. There were no lake-mesh size interactive effects either, indicating that macroinvertebrate effects were not apparent in any of the lakes regardless of nutrient concentrations and temperature. This general lack of significant effect contrasts with many reports from stream ecosystems (Irons et al., 1994; Greenwood et al., 2007; Boyero et al., 2016) and other studies on lakes (Tuchman 1993; Sabetta et al., 2000). There are a number of possible reasons for this lack of effect of macroinvertebrates including relative low biomass of these invertebrates and a more diverse group of benthic consumers that are less specialized to feed on terrestrial leaf material. Nonetheless, it thus appears that microbial detritivores, and not macroinvertebrates, are the primary agents of decomposition in boreal lakes in Ontario. As this result may be specific to our study region and vary with factors such as colonization time or leaf type (Greenwood et al., 2007; Ligeiro et al., 2010), the role of macroinvertebrates in leaf decomposition in lakes should nonetheless be examined more rigorously in the future.

Rates of C, N, and P release were all tightly coupled to mass-specific breakdown rates, which reflects a strong connection between nutrient release and detrital decomposition (Banks & Frost, 2017). Faster decomposition in warm, nutrient-rich lakes yielded higher flux rates of C, N, and P from decomposing detritus back into the water column as less of these nutrients were stored in the detritus. As much of the original leaf biomass was decomposed at higher breakdown rates, nutrient release ratios converged to ratios similar those of the original detrital biomass. This convergence happens because high loss rates lead to most mass being released from the original material, which means the ratios of loss must equal or be highly similar to the composition of the original material (Banks and Frost 2017). Slower decomposition rates in more northern lakes could result in more nutrient storage in the littoral zone simply due to less release

from organic breakdown and some uptake. In some instances, we found more N and P in litterbags than what was present at the beginning of the experiment indicating net uptake by microbial decomposers over the experimental duration. Where decomposition was relatively slower, ratios in released nutrients were thus more variable and residual leaf N and P concentrations appeared to be less determined by biomass breakdown and more related to microbial uptake and sequestration. As we used a common litter source in all lakes, differences in N:P release ratios from decomposing litter were entirely due to differences in the environment among our study lakes. This clearly shows that ratios of nutrient release from decomposing organic matter can vary substantially, even for a single leaf type, due to differences between nutrient incorporation by accumulating microbial biomass and release through material breakdown.

The stoichiometric ratios of released nutrients may also provide some indication about the relative supply of water column nutrients available to microbial detritivores for incorporation into accumulating biomass. For C:N ratios, we found release ratios (C:N 100-1000) well above that in the original leaf material (C:N 50), which indicates a disproportionate release of C relative to N. This elevated C:N release ratio indicates relatively low N supply from the water column to and N-limitation in colonizing detritivores. In terms of C:P ratios, we also documented elevated C:P release ratios in two regions, IISD-ELA and Kawartha Lakes, where we found the slowest and fastest rates of decomposition, respectively. We suspect that this reflects at least two separate processes controlling released C:P ratios: 1) low water column P concentrations constrain microbial biomass accrual, slow decomposition, and increase retention of P obtained from the leaf material and 2) high water P concentrations yield greater biomass of microbial decomposers, which acts as a nutrient sink for both internally and externally derived nutrients. In the other two

356 regions (Dorset and Kawartha Highlands), C:P ratios more closely matched, or were slightly
357 below, that in the original leaf litter. This indicates that there is no simple relationship between
358 released nutrient ratios, breakdown rates, and/or nutrient supply among the regions. We also
359 found some differences in N:P ratios released with those at IISD-ELA matching the original leaf
360 material and lower ratios in the other regions. It may be that relatively low N:P release ratios
361 reflect the relatively high N demand and N-rich cells of fungal decomposers in the three most
362 southern lake regions (Gulis et al., 2006). At the IISD-ELA, low water column N and P
363 concentrations may have limited microbial biomass (Gulis and Suberkropp, 2002), which
364 reduced the importance of microbial activity in controlling the uptake and release of these two
365 nutrients. Our results clearly demonstrate both regional and site-specific controls on the release
366 of nutrients back into the water column and that nutrient release ratios and ecosystem nutrient
367 cycling are a complex function of environmental factors. Given this, it appears that nutrient
368 release ratios are not entirely predictable from simple measures of initial detrital stoichiometry or
369 decomposition rates. Future work should better incorporate environmental controls of release
370 ratios into models predicting the effects of microbial mass on nutrient storage and release from
371 allochthonous materials (e.g., Webster et al., 2009).
372 Our results indicate that detrital processing may change in boreal lakes in response to
373 global change. Highly developed lakes in southern Ontario have experienced changes in nutrient
374 loading with possible increases or decreases in external P inputs (White, 2006). In addition,
375 global change scenarios forecast longer growing seasons and higher water temperatures for lakes
376 across Ontario (Minns et al., 2014). Altogether, warmer and more nutrient-rich lakes may
377 become more prevalent in Ontario, which would reduce the likelihood that allochthonous carbon
378 will be stored over longer time periods in lake sediments. At the same, increased decomposition

may increase nutrient release rates and constrain ratios of nutrient release from deposited leaf litter. Given the possible connections between detrital releases of C, N and P cycling and lake foodweb dynamics, future work should explore the implications of changes in decomposition and nutrient release ratios in northern lake ecosystems.

The results of this study provide a basis for understanding mechanisms that control leaf decomposition in the littoral zones of lakes. We found high variability in decomposition rates and nutrient release ratios from decomposing leaf litter among lakes in Ontario, Canada. This variability was largely related to differences in temperature and dissolved nutrient concentrations among lake regions. While we found no effects of macroinvertebrates (i.e., mesh size) on leaf breakdown rates, future studies should further examine whether detritus serves as a primary resource for littoral consumers and, if so, to identify the important shredder species influencing leaf breakdown in lake food webs. Such work would provide a better understanding of the role of external particulate carbon subsidies in benthic food webs and their influence on relationships between microorganisms and invertebrates. Given the high deposition rates of terrestrial leaf litter into temperate lake ecosystems, there is a clear need to extend the results of our study by more carefully examining mechanisms behind the processing of detritus in lakes.

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Figure Captions

Figure 1. Geographic location of our sampling sites within our four study regions (Kawartha Lakes, Kawartha Highlands, Dorset Lakes, and IISD-Experimental Lakes Area) in Ontario, Canada.

Figure 2. Breakdown rates (day^{-1}) from different lake regions for litterbags with macroinvertebrates (grey bars) or with macroinvertebrates excluded (white bars). Letters denote significant differences among regions. Shown are averages $\pm$ 95% confidence intervals from lakes within each region.

Figure 3. Linear relationships between decomposition rates (day^{-1}; log_{10} transformed) and A) total phosphorus ($\mu g\ L^{-1}$), B) temperature (°C), C) total dissolved nitrogen ($\mu g\ L^{-1}$), and D) pH. Lakes from different regions are denoted as: IISD-Experimental Lakes Area (open circle), Dorset Lakes (closed square), Kawartha Highlands (grey circle), and Kawartha Lakes (white triangle). Also note that IISD-ELA TDN values (25 $\mu g\ L^{-1}$) represent a median estimate as true values were below detection limit (50 $\mu g\ L^{-1}$).

Figure 4. The ratio of N- and P-specific fluxes and mass-specific decomposition rates (day^{-1}). Lakes from different regions are denoted as: IISD-Experimental Lakes Area (open circle), Dorset Lakes (closed square), Kawartha Highlands (grey circle), and Kawartha Lakes (white triangle).

Figure 5. Relationships between A) C- and N-, B) C- and P- and C) N- and P-release from decomposing litter. Positive values represent the quantity of element lost from the litterbag over

615 the incubation period. Lakes from different regions are denoted as: IISD-Experimental Lakes
616 Area (open circle), Dorset Lakes (closed square), Kawartha Highlands (grey circle), and
617 Kawartha Lakes (white triangle). Different elemental ratios are plotted with dotted lines whereas
618 the solid lines show the elemental ratio initially in the leaf litter.
619

620 **Table 1.** Location and water quality data for lakes studied in the IISD-Experimental Lakes Area,
621 Dorset Lakes, Kawartha Highlands, and Kawartha Lakes measured during the leaf litter
622 experiment. Shown are means values from multiple samples (water quality) or measured over the
623 course of the experiment (temperature). Lat. = latitude, Long. = longitude, TP = total phosphorus
624 (µg L^{-1}), DOC = dissolved organic carbon (mg L^{-1}), TDN = total dissolved nitrogen (µg L^{-1}),
625 Temp = temperature (°C), b.d. = below detection.
626

Region	Lake	Lat .	Long.	TP	pH	DOC	TDN	Temp.
IISD-ELA	L224	49.690	-93.715	10.42	6.95	3.31	b.d.	20.60
	L239	49.663	-93.722	11.43	7.05	7.84	b.d.	20.72
	L373	49.744	-93.799	12.22	7.20	4.11	b.d.	20.79
	L626	49.752	-93.799	11.06	7.00	4.81	b.d.	21.32
Dorset	Blue Chalk	45.199	-78.936	21.07	7.44	2.08	114.59	23.37
	Harp	45.379	-79.134	19.33	7.24	4.49	178.69	23.45
	Heeney	45.128	-79.102	20.23	5.99	3.31	-	24.20
Highlands	Coon	44.606	-78.199	20.47	7.93	6.54	85.76	24.57
	Long	44.690	-78.165	18.58	7.44	4.11	168.73	24.15
	Mississauga	44.711	-78.320	33.16	7.46	5.24	249.18	23.09
Kawarthas	Chemong	44.392	-78.391	32.25	8.33	5.47	390.25	23.69
	Pigeon	44.562	-78.503	32.78	8.48	4.89	331.62	23.71
	Stoney	44.553	-78.121	25.43	8.26	5.45	267.63	23.99
	Sturgeon	44.462	-78.712	30.74	8.44	5.30	348.59	24.03

627

628 **Table 2.** ANOVA results for analysis of decomposition rates within and among regions and due
629 to macroinvertebrate feeding. Degrees of freedom (df), type 3 sum of squares (SS), mean squares
630 (MS), f-ratio of mean squares (F), p-values, and r-squared values (r^2) are reported from a mixed-
631 effect general linear model. Non-significant difference (^{ns}) are denoted for Bonferroni corrected
632 *P*-values (0.05/4=0.0125).
633

Source	df	SS	MS	F	p-value	r^2
Region	3	0.00289	0.00096	11.42	<0.001	0.44
Macroinvertebrates	1	0.00042	0.00042	18.52	0.023^{ns}	0.06
Region*Macroinvertebrates	3	0.00007	0.00002	2.23	0.082^{ns}	0.01
Lake(Region)	42	0.00258	0.00007	6.90	<0.001	0.39
Total	49	0.00595	0.00013			0.90
Error	61	0.00061	0.00001			0.09

634
635

636 **Table 3.** Pairwise comparisons of stoichiometric release ratios across regions. Significant
637 differences in loss ratios are denoted by letters and were determined using Bonferroni corrected
638 t-test comparisons of elemental loss slopes ($p<0.013$).

Region	ΔC/ΔN	ΔC/ΔP	ΔN/ΔP
IISD-ELA	a	a	a
Dorset Lakes	b	a	ab
Kawartha Highlands	a	a	ab
Kawartha Lakes	b	b	b

639

Figure 1.

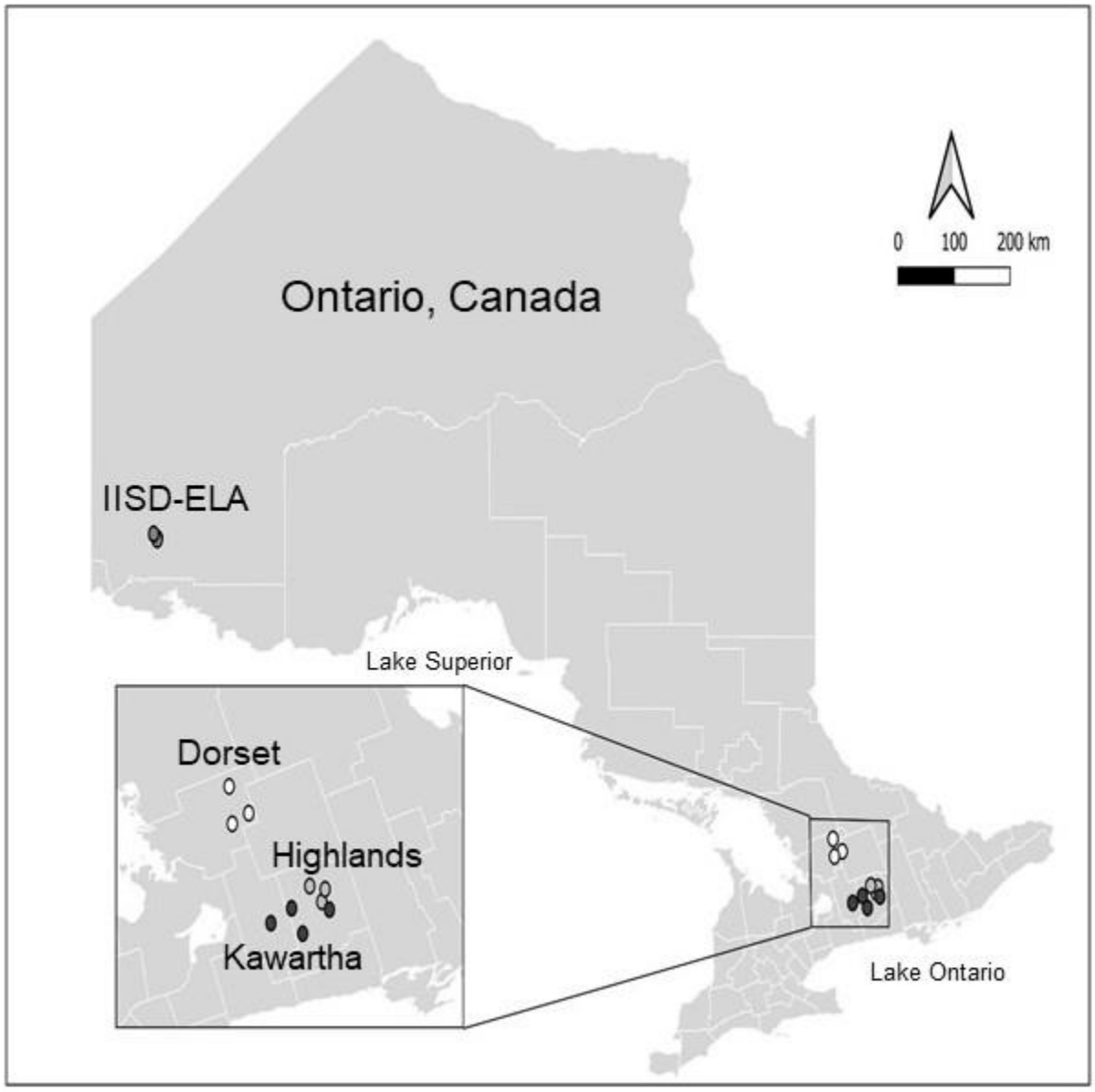

Ontario, Canada
0 100 200 km
IISD-ELA
Lake Superior
Dorset
Highlands
Kawartha
Lake Ontario

Figure 2.

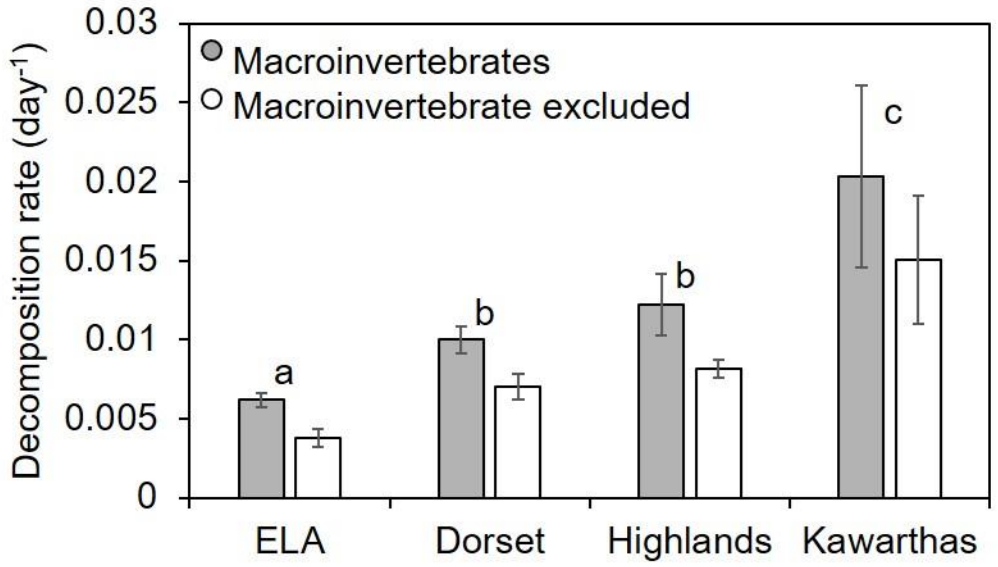

Decomposition rate (day-1)
0.03
0.025
0.02
0.015
0.01
0.005
0
Macroinvertebrates
Macroinvertebrate excluded
a
b
b
c
ELA
Dorset
Highlands
Kawarthas

Figure 3.

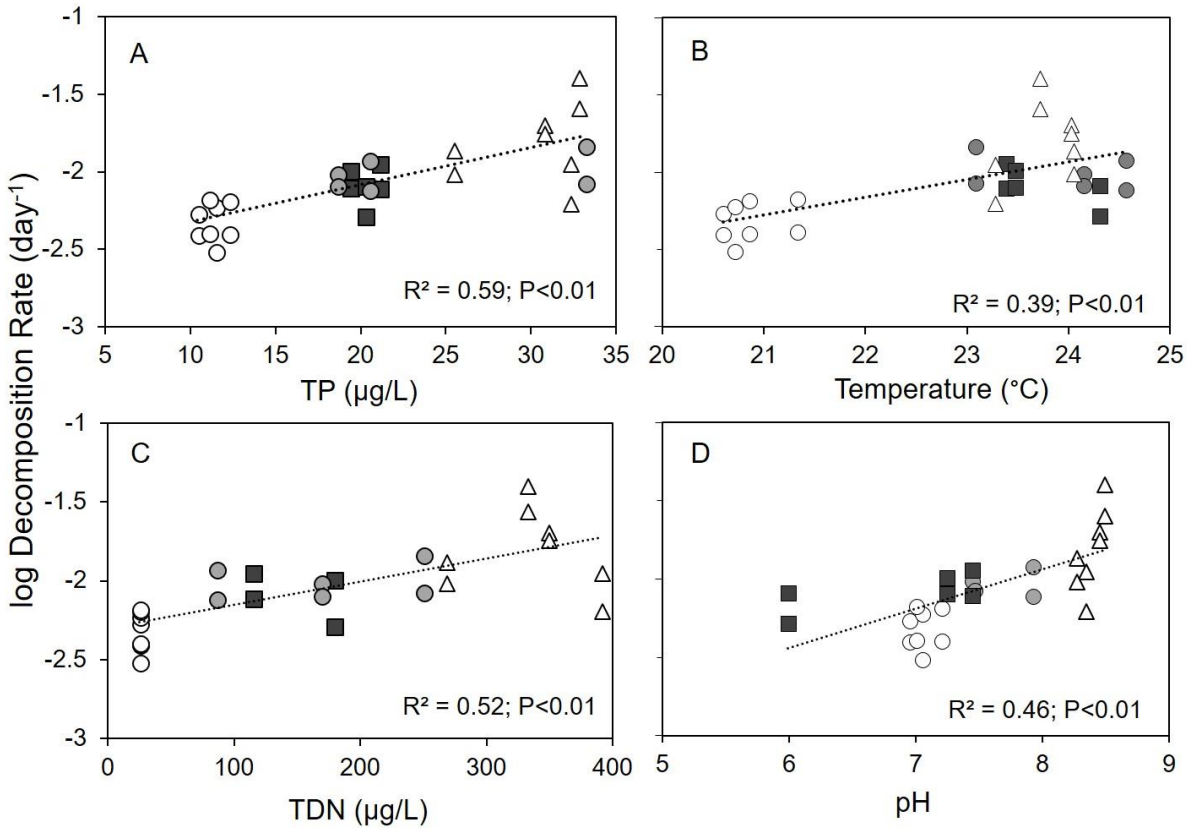

A
B
C
D
log Decomposition Rate (day-1)
TP (µg/L)
Temperature (°C)
TDN (µg/L)
pH
R² = 0.59; P<0.01
R² = 0.39; P<0.01
R² = 0.52; P<0.01
R² = 0.46; P<0.01

Figure 4.

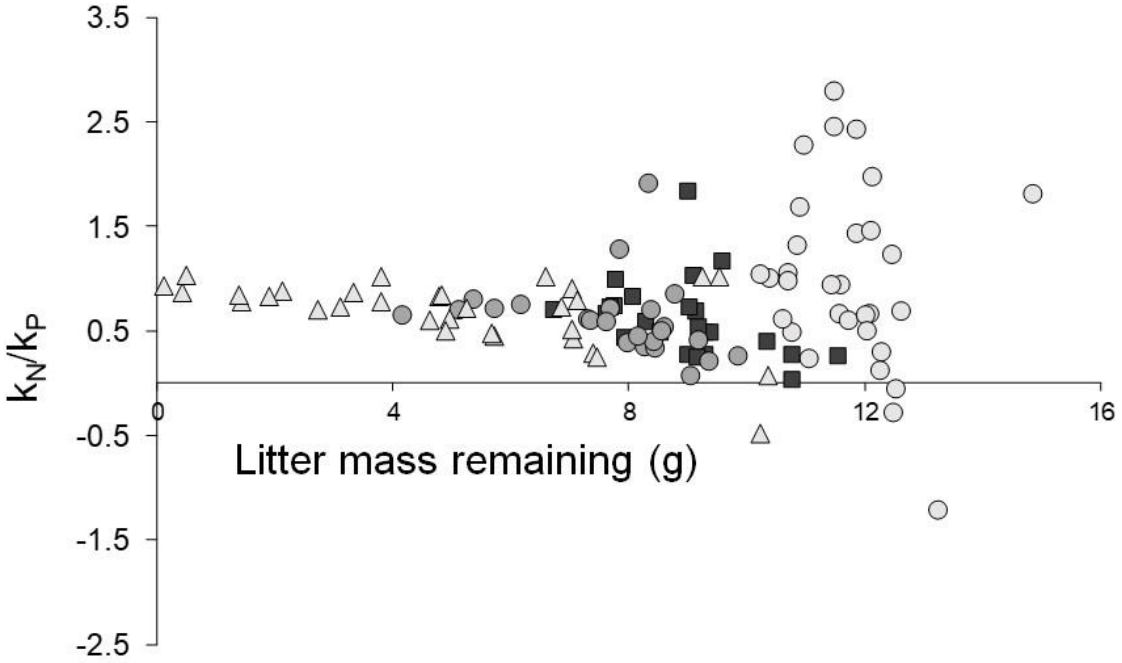
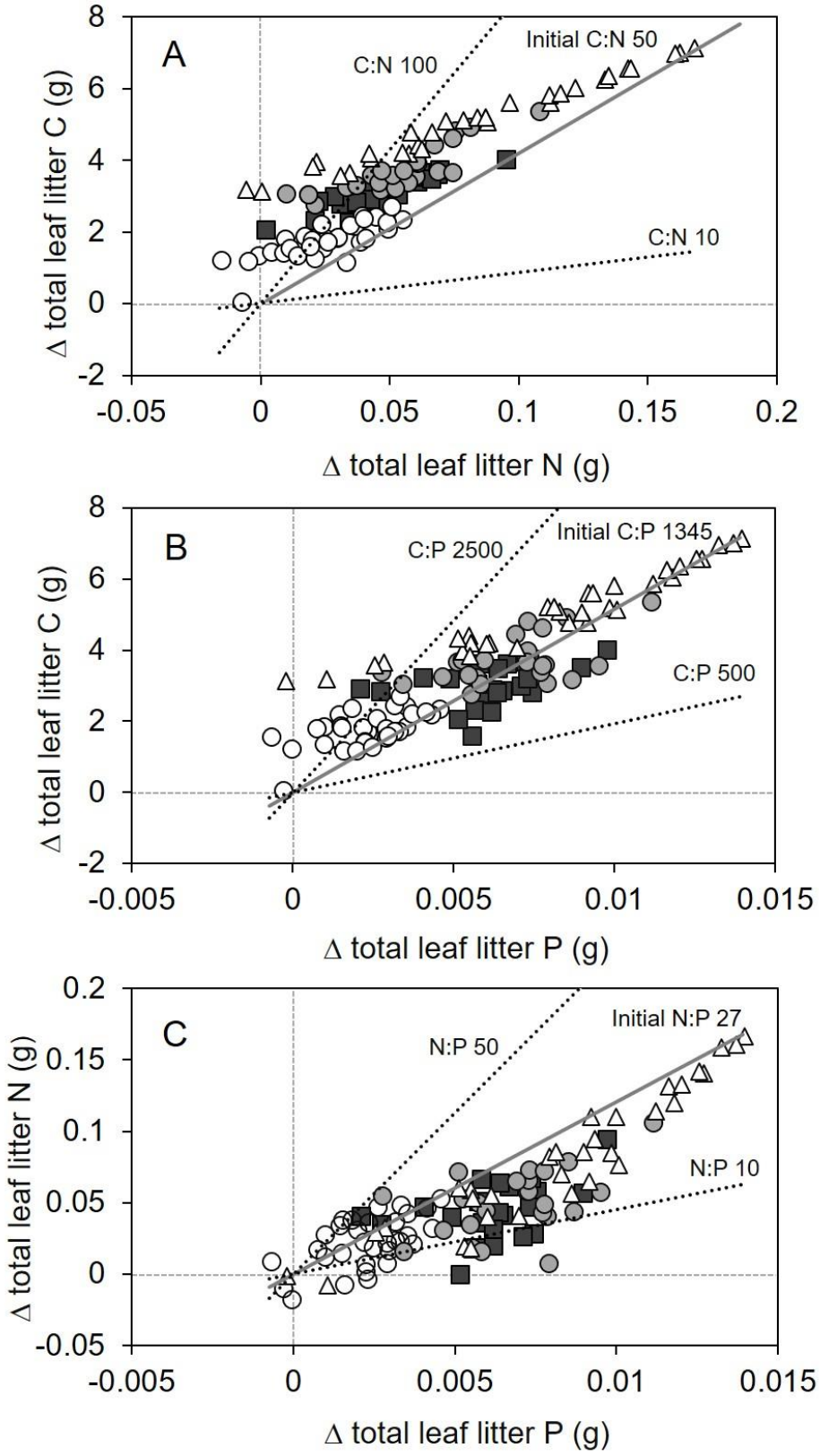


Figure 5.


A
C:N 100
Initial C:N 50
C:N 10
Δ total leaf litter C (g)
Δ total leaf litter N (g)
B
C:P 2500
Initial C:P 1345
C:P 500
Δ total leaf litter C (g)
Δ total leaf litter P (g)
C
N:P 50
Initial N:P 27
N:P 10
Δ total leaf litter N (g)
Δ total leaf litter P (g)