

Nutrient release rates and ratios by two stream detritivores fed leaf litter grown under elevated atmospheric CO₂

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With 5 figures and 1 table

Abstract: We examined how nutrient release by two common stream detritivores, *Asellus* and *Gammarus*, was affected by the consumption of aspen leaf litter from trees grown under elevated CO₂. We measured excretory release of dissolved organic carbon (DOC), ammonia (NH₄), and soluble reactive phosphorus (SRP) from consumers fed senesced leaves of *Populus tremuloides* (trembling aspen) trees grown under elevated (720 ppm) and ambient (360 ppm) CO₂. Contrary to predictions based on ecological stoichiometry, elevated CO₂ leaves caused greater NH₄ and SRP release from both animals but did not affect the release of DOC. Elevated CO₂ leaves reduced DOC : NH₄ and DOC : SRP ratios released from *Asellus* but did not affect these ratios from *Gammarus*. Both animals showed lower NH₄ : SRP release ratios after eating elevated CO₂ leaves. A mass balance model of consumer N and P release demonstrated that increased excretion rates likely resulted from reduced absorption efficiencies (and unchanged or higher digestive efficiencies) in these aquatic detritivores. Our results indicate that changes in leaf biochemistry resulting from elevated atmospheric CO₂ will strongly affect the ability of stream consumers to retain important biogenic elements. Increased release rates of NH₄ and SRP are another indication, along with reduced growth and reproduction, that litter produced under elevated CO₂ has strong effects on key physiological processes in detritivores with potentially strong consequences for nutrient cycling in streams of forested regions.

Key words: C : N : P ratios, detritivores, leaf litter quality, assimilation, ecological stoichiometry.

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Introduction

The atmospheric concentration of CO₂ is expected to rise to levels twice that of recent pre-industrial periods over the next fifty years (PRENTICE et al. 2001). Elevated atmospheric CO₂ concentrations strongly affect leaf biochemistry of many terrestrial plants (DRAKE et al. 1997, KÖRNER 2003). In addition, they increase the C:N and C:P ratios of leaves, especially when plants are grown under nutrient-limited conditions (KÖRNER 2003). These changes in green-leaf chemistry are partially maintained in the litter of some plants (NORBY et al. 2001) and can strongly affect soil invertebrates (e.g., COTRUFO et al. 1998). Less is known about how CO₂-mediated changes in litter will affect detritivorous invertebrates found in streams and rivers.

Leaf deposition can be a large percentage of annual organic matter inputs into small streams of the deciduous forest (e.g., FISHER & LIKENS 1973). This material link between terrestrial ecosystems and forested streams is one pathway by which increasing atmospheric CO₂ may alter ecological processes in forested streams (RIER et al. 2002, TUCHMAN et al. 2002, 2003). Litter from trees grown under elevated CO₂ concentrations can negatively affect the production of detritivorous bacteria and fungi in streams (RIER et al. 2002, TUCHMAN et al. 2002). Similarly, the growth of a detritivorous cranefly, *Tipula abdominalis*, was reduced by consumption of leaf litter that was produced under elevated atmospheric CO₂ concentrations (TUCHMAN et al. 2002). These results show that elevated CO₂ will potentially reduce the quality of leaf litter supporting decomposer production in stream food webs. How this reduced quality will affect other aspects of streams and their residents remains, however, largely unstudied. In particular, no work has examined how elevated CO₂ derived-litter will alter the rates and ratios of nutrient release by stream detritivores.

Alterations to leaf chemistry caused by elevated atmospheric CO₂ could have strong effects on nutrient release from stream invertebrates. High C:N and C:P ratios in food increase the assimilation efficiencies of N and P (respectively) and reduce their release rates from zooplankton consumers (ELSER & URABE 1999, FROST et al. 2004). High C:N and C:P ratios in food should also lower C assimilation as consumers work to free themselves of unneeded carbon (FROST et al. 2004, FROST et al. 2005). Based on a stoichiometric perspective, one would predict decreasing N and P release rates from detritivores eating nutrient-poor litter derived from plants grown at elevated CO₂ concentrations. Detrital biochemistry could also affect nutrient release by stream consumers through the effects that plant secondary compounds have on assimilatory processes, digestive and absorptive, in invertebrates (FELTON et al. 1992, GRAÇA & BARLOCHER 1998). For example, plant secondary compounds have been found at increased concentrations in leaves grown under elevated CO₂

(i. e., TUCHMAN et al. 2002, but see HARTLEY et al. 2000) and some of these (e. g., phenolics) are known to inhibit nutrient acquisition in invertebrates (FELTON et al. 1992, WATERMAN & MOLE 1994).

In this study, we examined how consumer nutrient release related to changes in leaf chemistry resulting from an elevated atmospheric CO₂ environment. We measured the release rates and ratios of dissolved organic carbon (DOC), ammonia (NH₄), and soluble reactive phosphorus (SRP) by two stream macroinvertebrates fed leaf litter produced by aspen trees grown under ambient (360 ppm) and elevated (720 ppm) atmospheric CO₂ concentrations. In addition, we used a mass balance model to examine the physiological mechanisms likely responsible for changes in nutrient release rates from invertebrates consuming leaf litter produced under elevated CO₂ concentrations. Our results indicate that the effects of atmospheric CO₂ concentrations on plant chemistry alter consumer nutrient release but the mechanisms accounting for changes in release rates and ratios remain an area in need of additional and directed study.

Methods

Production of Aspen leaf litter

Trembling aspen trees (*Populus tremuloides*) were grown under elevated (720 ppm) and ambient (360 ppm) atmospheric CO₂ conditions from leaf-out (May) through natural senescence (November) at the University of Michigan Biological Station (UMBS; Pellston, MI). All saplings were planted in open bottom root boxes containing a pre-mixed soil homogenate of 80% native rubicon sand amended with 20% topsoil to provide soil nutrients. During the treatment periods, all aspens were watered at least twice weekly. Clear plastic open-top chambers [1 m × 1 m × 2 m; see CURTIS & TEERI (1992) for design] were placed over sixteen cloned 4-year old saplings of *P. tremuloides*. CO₂ partial pressure was elevated in 8 of the 16 experimental chambers via manually adjusted flowmeters that dispensed 100% CO₂ into chamber input fans allowing for equal diffusion of CO₂ throughout the chamber (CURTIS & TEERI 1992). Chamber gas concentrations were monitored continuously in 8 elevated and 1 ambient CO₂ chamber by an infrared gas analyzer (LiCor model LI-6252) that logged data into a personal computer. Throughout autumnal leaf abscission, leaves were collected on a daily basis, air dried, and stored in airtight containers for both elemental analysis and use in detritivore experiments.

Invertebrate collection and pre-experiment feeding

We examined the effects of leaf type (ambient versus elevated CO₂) on the nutrient release rates and ratios of two stream consumers, *Asellus* and *Gammarus*. These invertebrates were chosen because they are two common stream detritivores in streams (GRAÇA 2001) and were readily collected from a small stream, Juday Creek, near the

University of Notre Dame (South Bend, Indiana). Invertebrates of the same size and developmental stage were sorted at the stream and placed in an insulated cooler containing stream water. In the laboratory, invertebrates were left in the dark and under aeration to allow gut clearance. After 24 hr, *Asellus* and *Gammarus* were transferred into plastic containers (1 liter each) containing one of the two types (elevated and ambient CO₂) of aspen leaf litter. Prior to this feeding, air-dried aspen leaves were soaked overnight in distilled water to allow rehydration and to remove leachable carbon substrates. Invertebrates consumed each food type for 48 hr at room temperature (20 °C) before the excretion experiment. Minimal animal mortality (<5 %) was found after this initial leaf consumption period. Consumption of leaf material by both invertebrates was visually confirmed by inspection of leaf litter and by the presence of fecal matter.

Excretion experiments

After the two days of feeding, animals were rinsed with distilled water and transferred in groups of eight to ten individuals into six replicate beakers containing 200 ml of distilled water. After 4 hr, water from excretion chambers was filtered through 0.2 µm polycarbonate filters that were pre-rinsed to remove potential organic contaminants (YORO et al. 1999). As excretion rates in animals held without food decline rapidly (VANNI 2002), nutrient release rates presented here are likely lower than those from animals feeding in streams. Samples of the distilled water from blank beakers having no animals present were also filtered and saved. All water was refrigerated at 4 °C until chemical analysis. Animals were also collected from each excretion beaker, dried at 60 °C for 24 hr, and stored frozen.

Chemical analyses

Excretion water was analyzed for DOC, NH₄, and SRP. We measured DOC with a Shimadzu TOC analyzer after acidification and purging of dissolved inorganic carbon (SHARP et al. 1993) within one week of the experiments. NH₄ was measured with a spectrophotometer using the phenate method (APHA 1992). SRP was measured with a spectrophotometer using the molybdate blue-ascorbic acid technique (APHA 1992). Both NH₄ and SRP were measured on the day following the experiment. Nutrient release rates were then calculated as the difference between post- and pre-excretion concentrations divided by the time and the collective mass of the excreting animals within one replicate. We weighed all animals on a Mettler microbalance after drying for 24 hr at 60 °C. In addition, we determined the C:N:P composition of the consumers and the two leaf food types. Carbon and nitrogen were measured on weighed subsamples with a Costech elemental analyzer (ECS 4010). Particulate phosphorus was assessed as above after samples were digested in boiling acid (leaf material) or in an autoclave with persulfate (animal material).

Nutrient release model

To examine the mechanisms potentially underlying nutrient release responses by consumers of litter grown under elevated CO₂, we derived a mass balance model that rela-

tes animal nutrient release rates to the digestion and assimilation of N and P (see STERNER 1997 and FROST et al. 2004 for details of similar mass balance models). As in FROST et al. (2004), we first delineated the C:N and C:P ratios under which C, N, and P are expected to limit consumer growth. As this preliminary analysis indicated that P should be in shortest supply in the leaf food (results not shown), we restricted further model development and analysis to a situation where P is assumed to be the limiting element for these consumers. Using this assumption, the rates of dissolved N and P release were calculated as:

$$\text{N release rate} = I_C \cdot \left(\frac{1}{f_{C:N}} \cdot D_N - \frac{1}{f_{C:P}} \cdot D_P \cdot A_P \cdot \frac{Q_N}{Q_P} \right) \quad (1)$$

$$\text{P release rate} = I_C \cdot \frac{1}{f_{C:P}} \cdot D_P \cdot (1 - A_P) \quad (2)$$

where I_C is mass-specific ingestion rate of C, D_N and D_P are the digestion efficiencies of N and P respectively, A_P is absorption efficiency of P, $f_{C:N}$ and $f_{C:P}$ are food C:N and C:P ratios respectively (both in mass units for the model), and Q_N and Q_P are the animal body contents of N and P respectively. We set ingestion rates in all model scenarios at a relatively high level for animals of this size ($I_C = 1 \text{ day}^{-1}$, MOLONEY & FIELD 1989) as the invertebrates in our experiment were fed *ad libitum*. In this model, we separated two key processes, digestion and absorption, underlying nutrient assimilation (WOODS & KINGSOLVER 1999). The digestion efficiency (D) is defined here as the fraction of ingested element broken into a dissolved form. Absorption efficiency (A) follows as the fraction of digested nutrients that move across the gut wall and is used for animal growth and metabolism. In this model, the product of the digestion and absorption efficiencies yields the assimilation efficiency of the animal. We used a range of food C:P ratios and C:N ratios similar to those found in the ambient and elevated CO_2 leaf litter (molar ratios of 1000–3000 and 30–60, respectively). D_P and A_P were varied in the model to determine how changes to consumer P digestive and absorption efficiencies would affect their N and P release rates.

Results

Nutrient release rates

Elevated CO_2 reduced the N and P content (and raised the C:N and C:P ratios) of leaf litter from aspen trees (Table 1). In addition, higher content of phenolics and lignins were found in litter produced under elevated CO_2 concentrations (Table 1). These differences in leaf chemistry did not significantly affect the release rate of DOC from either *Asellus* or *Gammarus* (Fig. 1). DOC release rates were significantly different between the two animal types as *Asellus* was found to have a higher release rate than *Gammarus* (Fig. 1). Food but not animal type significantly affected NH_4 release rates. In this case, NH_4 release rates were significantly higher from both stream consumers when fed on

Table 1. Chemical composition of leaves and consumers used in the nutrient excretion experiments. Shown are the means and standard deviations of replicate leaf and animal samples. Means and standard deviations calculated for leaf %C, %N, %P, C:N, C:P, and N:P ratios were derived from 13 separate leaves. 12 replicate leaves and 5 replicate leaves were analyzed for % total phenolics (% tot phen) and % lignin respectively. Means and standard deviations of consumers were derived from 8 replicate animal samples. Note that %C, %N, %P, % tot phen, and % lignin are all a percentage of dry mass and that all elemental ratios are molar. Data for leaf content of phenolics and lignin were taken from TUCHMAN et al. (2002).

	%C	%N	%P	C:N	C:P	N:P	% tot phen	% lignin
ambient CO ₂ aspen	48.2 ±1.51	2.17 ±0.35	0.067 ±0.01	28.3 ±3.75	1898 ±125	68.4 ±8.93	3.42 ±0.69	11.7 ±1.61
elevated CO ₂ aspen	48.7 ±0.76	1.14 ±0.32	0.03 ±0.01	58.85 ±7.16	3031 ±375	57.0 ±14.5	5.60 ±0.96	15.5 ±2.50
<i>Gammarus</i>	35.1 ±0.88	7.10 ±0.68	0.80 ±0.02	6.22 ±0.53	116 ±3.00	18.8 ±1.12		
<i>Asellus</i>	32.5 ±1.50	7.20 ±0.66	1.06 ±0.03	5.67 ±0.48	81.7 ±3.01	14.5 ±0.85		

elevated CO₂ aspen litter. Similarly, SRP release rates from both invertebrates were significantly increased by consumption of the elevated CO₂ litter (Fig. 1). Animal type also significantly affected SRP release rates. For both food types, *Gammarus* had significantly higher SRP release rates compared to *Asellus* (Fig. 1).

Nutrient release ratios

For both DOC:SRP and DOC:NH₄ release ratios, there was a significant interaction between leaf and animal type (Fig. 2). *Asellus* produced lower DOC:SRP and DOC:NH₄ ratios after consuming elevated aspen litter whereas this food did not affect these release ratios from *Gammarus* (Fig. 2). There was also a reduction of NH₄:SRP release ratios in both animals caused by consumption of elevated aspen food (Fig. 2). Reflecting its higher SRP release rates, *Gammarus* excreted significantly lower NH₄:SRP ratios than *Asellus* (Fig. 2).

Mass balance model

Changes in litter C:N and C:P ratios affected dissolved nutrient release by consumers in our mass balance model. Dissolved N release rates from consumers were predicted to increase with increasing C:P ratios in litter (Fig. 3). A simultaneous increase in litter C:N ratios should cause less N release from these consumers (Fig. 3), which is generally opposite to what we found in our release experiments. The mass balance model also showed that increasing lit-

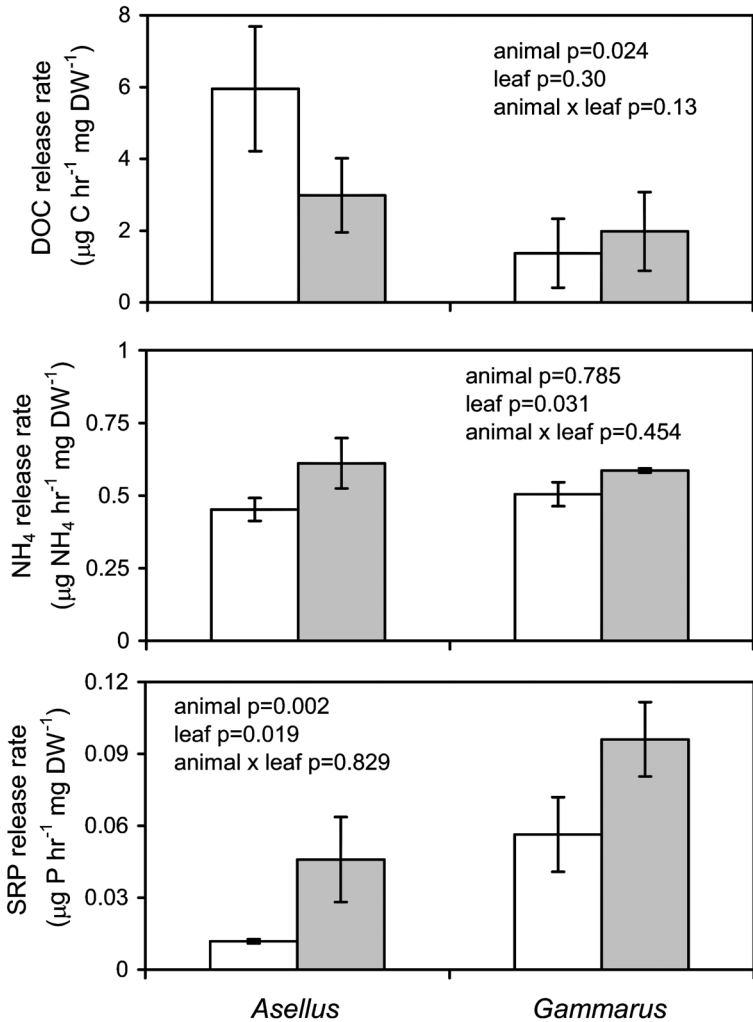


Fig. 1. Nutrient release rates from *Asellus* and *Gammarus* consuming aspen leaf litter grown under ambient (open bars) and elevated (closed bars) CO_2 . Shown are the mean and standard error of 6 replicate excretion jars. Also shown are the two-way ANOVA statistics with an interaction term run on JMP 3.2.

ter C:P ratios at fixed digestion and absorption efficiencies should lower dissolved P release by consumers (Fig. 3). As P was limiting in our model, food C:N ratio did not affect consumer P release rates. Changes to P digestion and absorption efficiencies in consumers were found to alter both N and P release in our model. Increasing digestion efficiencies of P caused more release of dissolved P but less dissolved N from consumers (Fig. 4) whereas increasing ab-

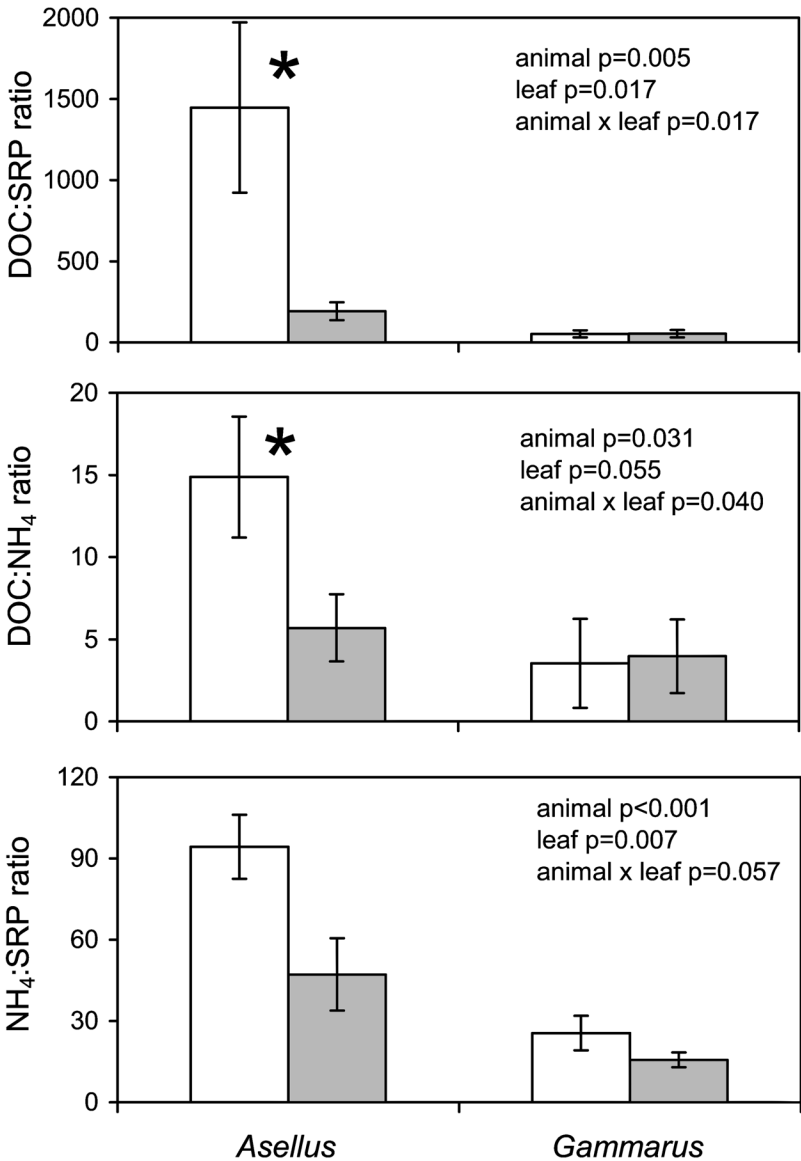


Fig. 2. Nutrient release ratios (all molar) from *Asellus* and *Gammarus* consuming aspen leaves grown under ambient (open bars) and elevated (closed bars) CO₂. Shown are the mean and standard error of 6 replicate excretion jars. Also shown are the two-way ANOVA statistics with an interaction term run on JMP 3.2. * Indicates a significant (p < 0.05) difference between the two food for *Asellus*.

sorption efficiencies of P caused lower release rates of both dissolved N and P (Fig. 5).

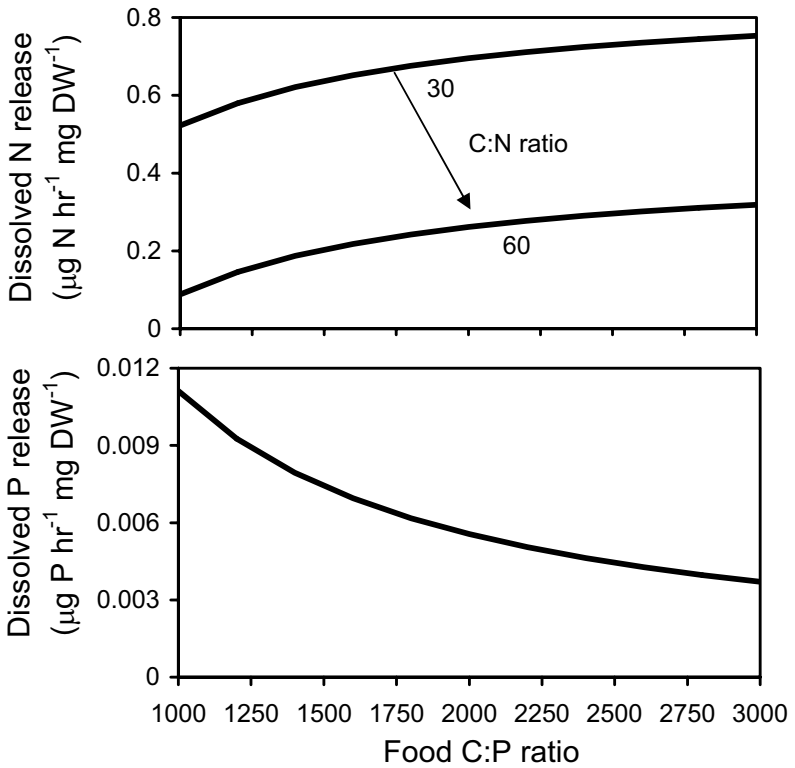


Fig. 3. Modelled dissolved N and P release rates from a consumer across a range of C:N and C:P ratios (by atoms) in food. A C:N ratio of 30 and C:P of 1900 roughly corresponds to ambient CO₂ litter, whereas a C:N ratio of 60 and a C:P ratio of 3000 were approximately those found in litter grown under elevated CO₂ in this study (see Table 1). Note that particulate nutrient release is not included in these rates. The digestion efficiency of N (D_N) and P (D_P) were held at 0.5 and the absorption efficiency of P (A_P) was held at 0.8.

Discussion

Our results show that leaf litter grown under elevated atmospheric CO₂ can alter nutrient release rates and ratios of two common freshwater detritivores. While elevated CO₂ leaf litter did not affect rates of DOC release, it increased the rate of NH₄ and SRP release from both consumers. These increased rates of NH₄ and SRP release occurred despite lower N and P content in elevated leaves. In addition, litter grown under elevated CO₂ altered the balance of elements released by *Asellus* and *Gammarus*. Release rates of SRP increased relatively more than NH₄, which resulted in lower NH₄:SRP ratios released from these consumers. It thus appears that increasing atmospheric CO₂ concentra-

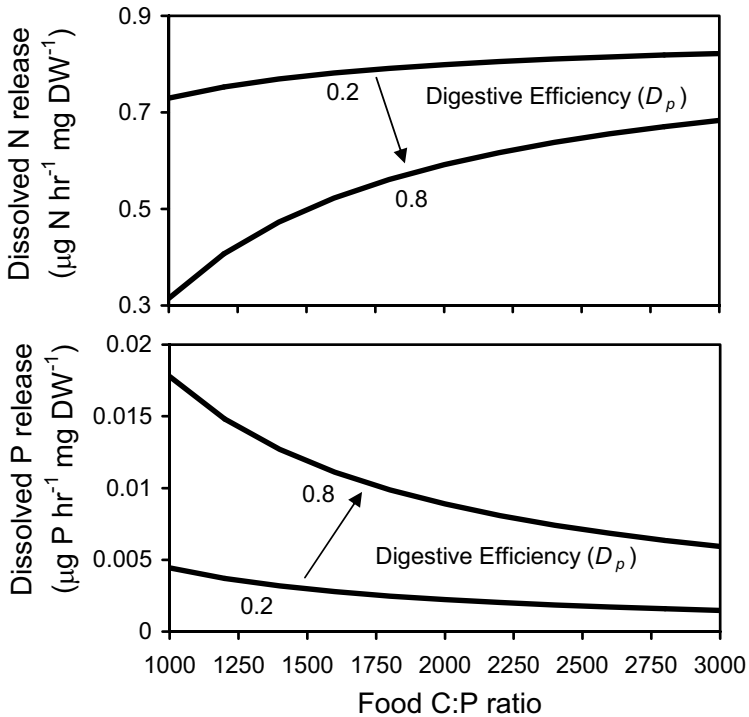


Fig. 4. Modelled dissolved N and P release rates from a consumer at contrasting digestion efficiencies (D_p) and a range of food C:P ratios (by atoms). Note that particulate nutrient release is not included in these rates. The digestion efficiency of N (D_N) was held constant at 0.5, the absorption efficiency of P (A_p) was kept at 0.8, and the C:N ratio of food was constant at 30.

tions through its effects on the quality of leaf litter have the potential to alter the nutrient dynamics of stream food webs.

Our results raise the question of why invertebrates did not increase DOC release but did increase NH_4 and SRP release when eating a food relatively more rich in C and poor in N and P. Particular physiological processes (e. g., respiration) may have limited the release of DOC from consumers eating litter having these relatively high C:N and C:P ratios (e. g., STERNER 1997, FROST et al. 2004). However, the precise mechanisms that constrained DOC release in our experiments remain to be determined. The increased NH_4 and SRP release rates that we observed in our experiment also do not conform to stoichiometric expectations, assuming fixed digestion and absorption efficiencies (Fig. 2). One potential cause of increased NH_4 and SRP release rates from detritivores in our experiment was the increased abundance of secondary carbon compounds (i. e., lignins and total phenolics) in elevated leaves. Such com-

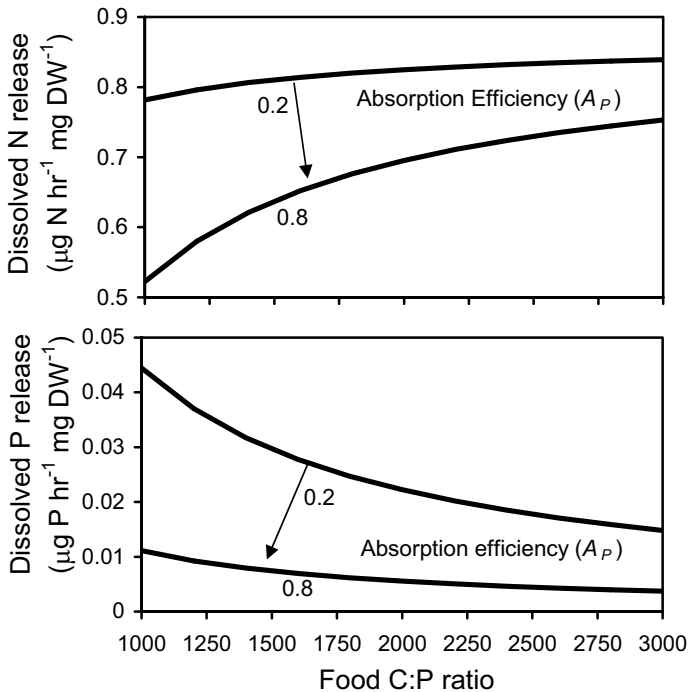


Fig. 5. Modelled dissolved N and P release rates from a consumer at contrasting absorption efficiencies (A_P) and a range of food C:P ratios (by atoms). Note that particulate nutrient release is not included in these rates. The digestion efficiency of N (D_N) and of P (D_P) were held constant at 0.5 and food C:N ratio was kept constant at 30.

pounds may slow invertebrate growth (e. g., TUCHMAN et al. 2003), which would presumably lower or eliminate demands for N and P. Another possible effect of higher phenolic content in elevated CO_2 detritus is internal physiological damage that reduces the ability of stream invertebrates to assimilate N and P. Phenolics in leaf litter are known to alter detritivore nutrient retention but the mechanisms responsible for these effects remain unclear (FELTON et al. 1992). An alternative explanation is that carbon assimilation was reduced by greater digestive resistance in the leaf litter and caused C-limitation of consumers. Reduced acquisition of C from elevated leaves would presumably lower the metabolic demand for N and P and increase their rates of release. However, reduced digestion would likely lead to less N and P excretion (not more as we observed) as more of these nutrients would have remained bound in the material being egested by the invertebrates.

Our mass balance model indicates that the increased nutrient release rates by invertebrates consuming elevated CO_2 detritus in this study are likely a

consequence of dramatically reduced absorption efficiencies (perhaps caused by the increased concentrations of secondary compounds). High rates of N and P excretion were found only to occur in the model when consumers were given a combination of high digestion and low absorption efficiencies. This situation results in most ingested material being converted into dissolved material but little of the digested material being incorporated into new body mass. Assuming these two physiological conditions (high digestion but low absorption efficiency) are not met, the only other potential explanation for high nutrient release rates from the stream invertebrates consuming elevated CO_2 derived litter is that they are releasing elements previously acquired and being lost from body tissues. Either case, very low absorption efficiency or net loss of elements, would undoubtedly be a dire situation for an invertebrate and place strong constraints on growth and reproduction. Future work should focus on determining how key physiological processes (ingestion, digestion, and absorption) involved in nutrient acquisition and retention are affected in aquatic invertebrates consuming food produced under ambient and elevated CO_2 concentrations.

The effects of leaves grown under elevated CO_2 on nutrient release from aquatic detritivores could have also been moderated by gut bacteria. *Asellus* has recently been shown to have significant quantities of midgut bacteria that presumably aid in the digestion of highly recalcitrant leaf material (ZIMMER & BARTHOLME 2003). Changes in leaf biochemistry produced by elevated CO_2 could negatively affect gut bacteria and thereby alter nutrient retention by this animal. Free-living, heterotrophic bacteria of the East Branch of the Maple River (northern Michigan) were negatively affected by leaf litter grown under elevated CO_2 (RIER et al. 2002). If gut bacteria and their digestive activities in detritivores were negatively affected, then particulate release of C, N, and P should increase whereas dissolved release should decrease from these invertebrates. Consequently, it would appear that other mechanisms are responsible for the increased dissolved N and P release from animals in our study. Nonetheless, the role of gut bacteria in nutrient retention and their responses to elevated CO_2 should be more carefully examined in the future.

Our results indicate that changes in litter biochemistry resulting from increasing atmospheric CO_2 will lead to greater N and P release by stream consumers. This increased nutrient release may reduce N and P limitation by stream microbial heterotrophs and/or autotrophs or alter the net retention of terrestrial nutrients in particular streams. However, the net effect of elevated CO_2 on stream nutrient cycling should perhaps be evaluated from a broader perspective. For example, leaf material produced under elevated CO_2 can result in greater mortality and reduced growth of stream macroinvertebrates (e. g., TUCHMAN et al. 2002, 2003). If less animal biomass contributed to the release of nutrients, the total flux of N and P moving through consumers

would be reduced. Less transformation of nutrients by stream detritivores would lead to greater proportions of N and P locked in leaf mass or an increased importance of nutrient processing by leaf-associated bacterial and fungal films, which are also strongly affected by the altered chemistry of this organic material (RIER et al. 2002). It is unclear how changes in the mass of litter deposited, another potential effect of elevated atmospheric CO₂ (AMTHOR 1995), or the mixing of detritus from multiple tree species (e. g., HÄTTENSCHWILER & BRETSCHER 2001) will alter these nutrient dynamics in streams. Both of these effects should be considered in future research. Regardless, our evidence indicates that elevated atmospheric CO₂ has the potential to strongly alter nutrient cycling in ecosystems by way of changes in the physiology of metazoan consumers. Consequently, nutrient dynamics at the plant-animal interface is an area in need of additional research if we are to fully understand the implications of increasing atmospheric CO₂ on ecological processes in aquatic ecosystems.

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