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RESEARCH ARTICLE

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Animal-mediated organic matter transformation: Aquatic insects as a source of microbially bioavailable organic nutrients and energy

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Abstract

1. Animal communities are essential drivers of energy and elemental flow in ecosystems. However, few studies have investigated the functional role of animals as sources of dissolved organic matter (DOM) and the subsequent utilization of that DOM by the microbial community.
2. In a small forested headwater stream, we tested the effects of taxonomy, feeding traits, and body size on the quality and quantity of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) excreted by aquatic insects. In addition, we conducted steady-state solute additions to estimate instream demand for labile C and compared it to the C excreted by invertebrates.
3. Individual excretion rates and excretion composition varied with body size, taxonomy and feeding guild. The estimated average community excretion rate was 1.31 μg DOC· per mg insect dry weight $(DW)^{-1}$ hr^{-1} and 0.33 μg DON·mg DW^{-1} hr^{-1}, and individuals excreted DON at nearly twice the rate of NH_4^+.
4. This DOM was 2–5 times more bioavailable to microbial heterotrophs than ambient stream water DOM.
5. We estimated that the insect community, conservatively, excreted 1.62 mg of bioavailable DOC·m^{-2} hr^{-1} and through steady-state additions measured an ambient labile C demand as 3.97 ± 0.67 mg C m^{-2} hr^{-1}. This suggests that insect-mediated transformation and excretion of labile DOC could satisfy a significant fraction (40 ± 7%) of labile C demand in this small stream.
6. Collectively, our results suggest that animal excretion plays an essential functional role in transforming organic matter into microbially bioavailable forms and may satisfy a variable but significant portion of microbial demand for labile C and N.

KEYWORDS
biogeochemical cycling, brown food web, consumer nutrient dynamics, dissolved organic carbon, dissolved organic nitrogen, microbial ecosystem, organic matter decomposition

1 | INTRODUCTION

Research at the interface of community and ecosystem ecology increasingly seeks to understand the functional role animals play in biogeochemical cycles (Metcalfe et al., 2014; Schmitz et al., 2014; Yang & Gratton, 2014; Zou, Thébault, Lacroix, & Barot, 2016). The abundance, biomass, and functional and taxonomic compositions of animal communities can shape the stoichiometry, rates

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and magnitudes of elemental flows through ecosystems via consumer-driven nutrient cycling, which can, in turn, influence food web dynamics (Atkinson, Capps, Rugenski, & Vanni, 2017; Roman & McCarthy, 2010; Vanni, 2002). Inorganic nutrient recycling inextricably links the trophic dynamics of green or primary production-based food webs with those of brown or detritus-based food webs (Daufresne, Lacroix, Benhaim, & Loreau, 2008; Pace, Cole, Carpenter, & Kitchell, 1999; Zou et al., 2016). In contrast, the magnitude of animal-mediated flows of organic nutrients and organic energy and the functional roles these play in ecosystem-level processes are understudied. Importantly, animal metabolism mediates the ontogenic transformation of food resources, producing particulate and dissolved organic wastes (Cherif & Loreau, 2013; Jumars, Penry, Baross, Perry, & Frost, 1989; Moore et al., 2004)—the latter of which may support further microbial production (Jumars et al., 1989; Nagata, 2000). Thus, organic "wastes" produced by animals may represent a significant biogeochemical flux of labile organic matter that further links green and brown food webs.

Insects are key detritivores in many ecosystems and, after microbes, are often the most ubiquitous and abundant animals and critical drivers of ecosystem function (Yang & Gratton, 2014). In streams, insects process large quantities of living and detrital organic matter, and accelerate decomposition (Wallace & Webster, 1996). The use of different food resources depends, in part, on an individual's feeding guild (functional feeding group (FFG; Cross, Wallace, & Rosemond, 2007). For instance, shredders (vascular plant tissues and some microbes) and predators (consuming other animals) have distinct resource bases, while collectors (gatherers and filterers) and scrapers share a resource base (microbes, algae and detritus). Some aquatic insects can satisfy >20% and up to 100% of their C demands by consuming bacteria (Collins, Sparks, Thomas, Wheatley, & Flecker, 2016; Hall & Meyer, 1998).

Growth of microbial heterotrophs (hereafter "microbes") may be limited or co-limited by organic energy (labile C) or nutrient (N, P) availability (Daufresne et al., 2008; Sinsabaugh, Hill, & Follstad Shah, 2009). Much of this resource demand is supplied by dissolved organic matter (DOM; Jumars et al., 1989; Meyer, 1994)—a complex mixture of molecules with elemental stoichiometries dominated by C, H, O, N, P and S. In marine and freshwater ecosystems, <20% of the ambient DOC is typically bioavailable to heterotrophic microbes (Søndergaard & Middelboe, 1995), most likely leaving them carbon-limited or carbon-nutrients co-limited (Bernhardt & Likens, 2002; Daufresne et al., 2008; Sinsabaugh, Follstad Shah, Hill, & Elonen, 2012). C-limitation of microbial growth provides an important control within brown food webs and between brown and green food webs enhancing nutrient availability and promoting mutualistic plant–microbe interactions (Daufresne & Loreau, 2001; Zou et al., 2016).

Research in animal-mediated organic matter transformation highlights the controls on (Allgeier, Wenger, Rosemond, Schindler, & Layman, 2015; Vanni & McIntyre, 2016) and ecosystem effects of (Atkinson et al., 2017; Metcalfe et al., 2014; Sitters et al., 2017) inorganic nutrient recycling. As such, animal influences on inorganic nutrient cycling are more effectively integrated into biogeochemical and food web models than are animal-mediated impacts on organic nutrient dynamics and the flow of energy (Atkinson et al., 2017; Zou et al., 2016). Though limited, evidence suggests that a portion of ingested resources are excreted as DOC (James, Xenopoulos, Wilson, & Frost, 2007; Meyer & O'Hop, 1983), which may be left more bioavailable due to physical (e.g., fragmentation) and chemical (enzymatic breakdown of macromolecules) digestive processes (Jumars et al., 1989; Metcalfe et al., 2014; Moore et al., 2004). In a detritus-based ecosystem, addition of labile carbon stimulated microbial production and subsequently enhanced detritivore production (Wilcox, Bruce Wallace, Meyer, & Benstead, 2005). Thus, microbes utilizing labile organic excreta and from animals feeding in the green or brown food webs may provide a positive feedback on animals feeding on brown food web microbes.

Understanding the roles of insects in producing microbially bioavailable energy and nutrients from organic matter is important for predicting how biogeochemical processes and food web dynamics respond to anthropogenic perturbations that negatively impact insect abundance (Hallmann et al., 2017) and diversity (Dirzo et al., 2014), and that produce shifts in insect community composition (Hawkins & Yuan, 2016). To illuminate the role of animals in shaping the quantity, chemical composition and bioavailability of DOM in streams, we focused on insects and asked two questions: (a) How do taxonomy and trophic feeding guilds affect the rate and composition of DOM excreted by aquatic insects? (b) Does DOM excreted by insects provide an energy and/or nutrient subsidy to heterotrophic microbes? We investigated these questions through field excretion incubations measuring the rate and composition of DOM excretion, and laboratory experiments measuring the microbial degradation of insect-excreted DOM.

2 | MATERIALS AND METHODS

The Fair Hill Experimental Watershed (39.718° N, 75.835° W) is a well-studied close-canopied headwater stream watershed with first- and second-order subwatersheds in the Northern Piedmont ecoregion of Maryland, United States of America. The mean annual water temperature is 12.6°C and ranges from 0°C during the winter to 24°C (S. P. Inamdar, unpublished data). The watershed is dominated by a mature deciduous forest comprised of *Fagus grandifolia* (American beech), *Liriodendron tulipifera* (yellow poplar) and *Acer rubrum* (red maple), with a strip of grassland around the perimeter (see Supporting Information Figure S1). Soils are predominantly inceptisols over gneiss and schist bedrock. Physicochemical stream water parameters including dissolved oxygen (DO) and temperature were measured at the second-order watershed outlet using a YSI Exo2 Sonde (Supporting Information Table S1).

2.1 | Field excretion incubations

Excretion incubations were conducted in the autumn of 2015 (October) and spring of 2016 (March–April). We focused on abundant and readily collectable insect taxa. We collected insects from riffle and run habitats using a kick net (500 μm), disturbing the substrate by hand, and sorting individuals into a compartmented tray filled with filtered stream water. To ensure a measurable response, small-bodied individuals of the same morphotype were combined (n = 2–10). Trichoptera were not removed from their cases prior to the start of the incubations.

We filtered stream water to 0.7 μm (GF/F) and added 35 ml to acid-washed, ashed and pre-rinsed amber glass vials. Ten per cent of prepared vials were reserved for no-insect controls. Insects were placed in the remaining vials and allowed to excrete at ambient stream temperatures (11–15°C; Supporting Information Table S1) for approximately 2.5 hr in the fall and 4 hr in the spring. Four hours is longer than most insect excretion incubations measuring nutrients (0.5–2 hr; Vanni & McIntyre, 2016), and excretion rates decline in animals held without food (Vanni, 2002); however, this duration was necessary to achieve a sufficient (>10%) increase in excreted DOC concentration relative to background DOC concentration. At the end of the incubation, the water in all vials was re-filtered (0.7 μm GF/F) into fresh vials and individuals were preserved in 20 ml of 85% ethanol in a plastic centrifuge tube. Insects were identified to the genus level and FFGs assigned (Merritt, Cummins, & Berg, 2008). For 99% of incubations, sorting individuals by morphotype generated a single genus, the three vials with multiple genera were excluded from further analysis. Ten blank vials were also prepared to correct for mass residue from the preservation solution and leaching from the vial. To estimate insect dry weight (DW), we dried the entire contents (ethanol and insect) of the sample preservation vial at 50°C in an aluminium tin and weighed it (±0.01 mg). Drying of the blank vials indicated 1.50 ± 0.01 mg of residue in the preservation solution. All insect weights were corrected for this mass. All excretion rates are reported as mass-specific excretion rates: μg DOC, DON or NH_4^+ per mg insect DW per hour. To estimate community excretion, we applied our excretion estimates to areal biomass and biomass-weighted FFG composition from previously published work in other similar systems (Supporting Information Table S2).

2.2 | Biodegradable dissolved organic matter assays

To characterize the bioavailability of dissolved organic carbon (BDOC) and nitrogen (BDON) excreted by each functional feeding group, we conducted 28-day dark incubations and measured bioavailability as the fraction of DOC or DON lost. Controls consisted of deionized (DI) water controls and ambient stream water controls. Additionally, to alleviate potential nutrient limitation on ambient DOC bioavailability and to control for the excretion of inorganic nutrients and subsequent potential nutrient priming of DOC in excretion vials, a set of ambient stream water controls were amended with NO_3NH_4 and KH_2PO_4 in a 5:1 N:P molar ratio (~960 μg N L^{-1} and ~425 μg P L^{-1}), which reflects the stoichiometries of heterotrophic microbial biomass (7:1, Cleveland & Liptzin, 2007) and inorganic nutrient excretion by insects (3.7:1, Vanni & McIntyre, 2016). For insect-excreted BDOC, due to the high sample volume required, we separated individuals by order (Ephemeroptera, Plecoptera (non-shredding), Trichoptera, genus in the cases of *Tipula* and *Hexatoma*) and functional feeding group. Briefly, approximately 20 individuals (10 in the case of *Tipula* or *Hexatoma*) were placed into 1-L jars filled with approximately 750 ml of stream water pre-filtered through 0.7-μm glass fibre filters. For most groups (i.e., not *Hexatoma*), duplicate jars were prepared. The insects were then allowed to excrete for up to 4 hr. The contents of each jar were then filtered through 0.7-μm filters into six replicate 40-ml amber glass vials. We used three vials to measure initial chemical and optical characteristics. The remaining vials were incubated at ~20°C for 28 days and then analysed.

2.3 | Net DOC uptake and spiralling

To characterize net ambient microbial demand for labile C in our system and compare it to the DOC flux supplied by macroinvertebrates, we conducted a series of steady-state solute additions in September and October 2015. Briefly (full details in Supporting Information Appendix S1), a fluid metering pump (FMI International, model QBG2) injected a mixture of NaCl (conservative tracer) and labile DOC (acetate, pyruvate, citrate or glucose) at the top of a 225 m reach and samples were collected at regular intervals downstream. We then calculated spiralling metrics as the loss of DOC relative to conservative tracer.

Uptake length (S_w, m) was calculated as the inverse of the slope (k, m^{-1}) of the downstream decrease in DOC concentration:

$$\ln(\mathrm{DOC}_d) = \ln(\mathrm{DOC}_0) - k \times d \quad (1)$$

where DOC_0 is the DOC concentration at the point of injection, and DOC_d is the downstream DOC concentration at distance d in metres. DOC concentration data were corrected for lateral inflow using background corrected Cl^- concentrations measured from a field-prepared standard curve relating specific conductance at 25°C to the Cl^- concentration. The uptake velocity (v_f; m/s, units reported in text as mm/min) was calculated as:

$$v_f = (Q \div w) \div S_w \quad (2)$$

where Q (m^3/s) is the discharge, and w (m) is the average width (0.73 m) over the 225 m reach. The use of highly labile monomers (e.g., acetate and glucose) and plant leachates typically overestimates the ambient v_f and areal uptake (U) of DOC (Mineau et al., 2016; further discussion in Supporting Information Appendix S1). To better estimate ambient areal uptake, we adopt the methods of Mineau et al. (2016) and assume that the actively cycling carbon pool consists entirely of BDOC and that the v_f estimated by labile compound additions is accurate for the labile pool. Thus, we calculate

areal uptake U_{BDOC} (mg m^{-2} s^{-1}, units reported in text as mg m^{-2} hr^{-1}) as:

$$U_{BDOC} = v_f \times BDOC \quad (3)$$

where BDOC is the concentration of bioavailable DOC (mg/m^3) as determined from BDOC assays conducted during invertebrate sampling.

2.4 | Chemical and optical analyses

All excretion and bioavailability samples and controls were analysed for DOC, total nitrogen (TN), ammonium (NH_4^+), nitrate (NO_3^-) and DOM fluorescence. We used a Shimadzu TOC-L to measure DOC and TN. Ammonium and nitrate were analysed on a Seal Analytical AQ2 discrete nutrient analyser using the salicylate and cadmium reduction methods, respectively. For excretion samples, DON was calculated as the difference between total and inorganic N. For bioavailability incubations, due to the potential for microbial DON production from inorganic N, we calculated the DON concentration as a function of tryptophan-like fluorescence with a regression developed from using a subset of similar taxa from the excretion incubation ($DON = 0.81 \times TRP^{0.74}$; $R^2 = 0.92$). Steady-state additions were only analysed for DOC. All samples were refrigerated at 4°C and analysed within 48 hr.

We measured DOM fluorescence to understand the effects of insect taxonomy and feeding traits on DOM composition and its bioavailability to microbes. DOM fluorescence excitation–emission matrices (EEMs) and UV-Vis absorbance were measured simultaneously on a Horiba Aqualog fluorometer in a 1-cm quartz cuvette. We collected EEMs by measuring, at each excitation wavelength from 550 to 238 nm (4 nm increments), the fluorescence emission spectrum between 250 and 827 nm (4 nm increments). If the UV-Vis absorbance of EEMs was >0.2 A at 254 nm, they were diluted to <0.2 A using carbon-free DI water so that the inner filter effect correction would be valid (Ohno, 2002). All EEMs were DI blank-subtracted, instrumental bias, inner filter effect, and dilution-corrected.

2.5 | DOM composition modelling and statistical analyses

The resulting fluorescence EEMs were analysed using parallel factor analysis (PARAFAC) modelling. PARAFAC is a statistical procedure that decomposes the large volume of data contained in an EEM into a few (typically 3–13) statistically unique fluorescence "components" (DREEM; Murphy, Stedmon, Graeber, & Bro, 2013). These components are broadly associated with unique chemical structures or compounds (Cory & McKnight, 2005). These components represent complex mixtures of biomolecules and are frequently described as protein-like (tryptophan or tyrosine), humic-like and fulvic-like fluorescence. The abundance of protein-like fluorescence is often a correlated with higher concentrations of highly labile BDOC (Cory & Kaplan, 2012).

The EEMs from this study were combined with surface water EEMs from ongoing research at the Fair Hill watershed for a total of 1,368 EEMs used in the model. The final model was validated using split-half validation (Murphy et al., 2013). Fluorescence data were reported as "concentrations" (F_{max}, Raman Units, RU) and "compositions" ($\%F_{max}$, for a given sample described by a PARAFAC model with *n* components (abbreviated *E* in this study), the $\%F_{max}$ of the *i*th *E* is $\%F_{max\,E_i} = F_{max\,E_i}/(F_{max\,E_1} + \ldots + F_{max\,E_n}) \times 100$).

All statistical analyses were conducted in [R] 3.4.2 (R Core Team, 2017). We analysed relationships between excretion rates of BDOC, DOC, DON, and DOC:DON and fluorescence components using ordinary least squares regression. We assessed the relative influences of body size (metabolism), feeding guild (diet) and taxonomy, and their interactions on the excretion rates of DOC and DON and DOC:DON excretion composition using ANCOVA. To understand the effects of FFG and genus on the composition of DOC excreted, we ordinated fluorescent DOM composition using non-metric multidimensional scaling (NMDS, *metaMDS* function in the *vegan* package, Oksanen et al., 2017) with Bray–Curtis distances. We then used permutational analysis of variance (PERMANOVA, 9,999 permutations, *adonis*; Oksanen et al., 2017) with Bray–Curtis distances to quantitatively test patterns observed in the NMDS and (*betadisper;* Oksanen et al., 2017) to test for heterogeneity of variance among groups. Among-group differences were investigated with a pairwise post hoc PERMANOVA (*pairwise.perm.MANOVA* function in the *RVAideMemoire* package; Hervé, 2018).

3 | RESULTS

3.1 | Excretion rates

Mass-specific DOM excretion rates varied as a function of body size (Figure 1), diet (Figure 2b,d) and taxon (Figure 2a,c). These three variables explained 55% of the variance in DOC excretion rate ($F_{24,209} = 12.8$), 57% in DON rate ($F_{24,209} = 13.6$) and 52% in DOC:DON ($F_{24,209} = 11.5$; Table 1). Excretion rates decreased with increasing body size for DOC ($R^2 = 0.09$, $p < 0.05$) and DON ($R^2 = 0.31$ $p < 0.05$). At the community scale in a headwater stream, we estimated that the average insect community excreted 1.31 μg DOC·mg DW^{-1} hr^{-1} and 0.33 μg DON·mg DW^{-1} hr^{-1} (FFG biomass weighted, Supporting Information Table S2). Interestingly, our data showed that excretion of nitrogen as DON typically exceeded nitrogen as NH_4^+. The average individual DON:NH_4^+ ratio (molar as N) was 1.9 ± 0.2 and decreased in the order collectors (2.9 ± 1.2)>shredders (2.7 ± 1.0) >predators (1.7 ± 0.4) >scrapers (1.3 ± 0.3; Supporting Information Table S5). Functional feeding group influenced the excreted DOC:DON stoichiometry: ambient stream water > shredders > collectors > scrapers > predators (Figure 2f). This progression appears to reflect a food resource stoichiometry continuum from diets dominated by high C:N plant material (>20; Evans-White, Stelzer, & Lamberti, 2005) in shredders to increasing proportions of lower C:N microbial biomass (~7; Cleveland & Liptzin, 2007) in collectors and scrapers, with predators feeding on other low C:N insects (~4–6; Evans-White et al., 2005).

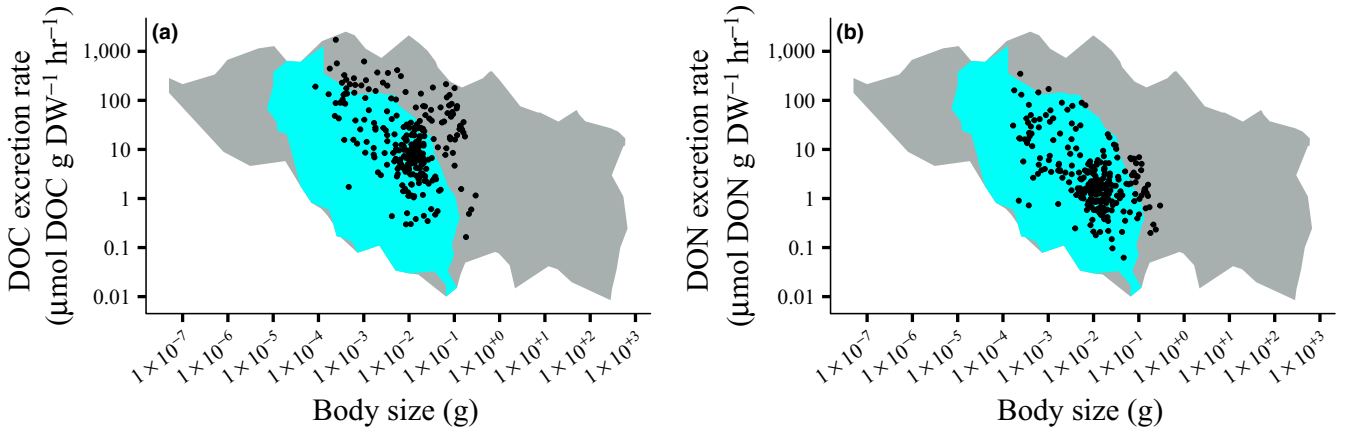

FIGURE 1 Excretion rates of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) as a function of body mass. For reference purposes, the data spaces for ammonia excretion complied by Vanni and McIntyre (2016) are presented for all animals (grey) and for insects only (blue)

3.2 | Excretion composition

PARAFAC modelling explained surface water and excreted DOM fluorescence in the EEMs and identified 10 fluorescence components (E1–E10, Supporting Information Figure S2). Components similar to eight of these components (E1–7 and 10) have been previously observed in the surface and ground waters of the Fair Hill watershed and were attributed to plant, soil and microbial sources (Singh, Inamdar, Mitchell, & McHale, 2014). Components resembling E4, 7 and 10 were previously described as fulvic acids, E1–3 and 6 humic acids, and E5 tryptophan (Supporting Information Figure S2).

Insects were also a source of some of these compounds. They excreted tryptophan (E5), fulvic acid-like compounds (E4, 7 and 10) and novel protein-like components E8 and E9. Component E9 strongly resembled tyrosine (Cory & McKnight, 2005), and this constituted a relatively higher proportion of excretion from the predator *Hexatoma* (Figure 2). E8 bore some similarities to tryptophan (E5). Increasing proportion of excretion resembling these two components was characteristic of shredder, collector and scraper excretions (Figure 3). All components were positively correlated with DOC and DON excretion for at least one insect taxon. For most insect taxa, these correlations were strongest for the protein-like components (E5, 8, 9; $R^2 \sim 0.90$, $p < 0.05$; Supporting Information Tables S3 and S4).

PERMANOVA of DOM composition by taxa and FFGs supported the ordination with significant differences among some taxa and FFG ($p < 0.05$). Post hoc tests of the FFG centroids suggested that the compositions excreted by each group were significantly different ($p < 0.05$), except between collector-filterers and collector-gatherers and between collector-gatherers and scrapers). Beta dispersion was also detected but was confined to taxa and FFGs with both location and dispersion effects.

3.3 | Excretion bioavailability

Dissolved organic carbon derived from insect excretion was up to five times more labile than ambient stream water DOC, and DON derived from excretion was up to twice as labile. Ambient bioavailabilities of DOC (17.5, 19.3%) and DON (48.7%, 50.2%) were similar in the autumn and spring, respectively. While ambient BDOC was not affected by nutrient amendment, ambient BDON decreased to 36% in nutrient-amended stream water controls. The BDOC content of insect excretions ranged from 35% to 88% (Figure 4), and BDOM content ranged from 54% to 83%. Excreted BDOC may have had some seasonal variation for the dipteran *Tipula* and trichopterans with mineral cases. In the autumn, BDOC was similarly high (~88%) among Plecoptera (*Eccoptura*, predators) and Trichoptera (collectors) and somewhat lower in the obligate shredder *Tipula* (63%). In the spring, BDOC in predators (*Eccoptura*) remained high (~82%), while collectors, scrapers and shredders (Ephemeroptera and Trichoptera) exhibited lower BDOC ~47%–68%, and BDOC from the obligate shredder *Tipula* was only 35% (Figure 4). Similarly, excreted BDON was higher in the autumn (82% ± 1%) and lower in the spring (65% ± 3%), but bioavailability for all taxa decreased similarly.

A PCA of DOM fluorescence composition before and after biodegradation provided further insight into the microbial biodegradation dynamics of excreted DOM and potential taxon or trait-dependent responses. Most of the variation (71%) in the fluorescence bioavailability of excreta was explained by degradation of protein-like fluorescence (primarily E5 and E9, secondarily E8; Figure 4, PC1). Dynamics in the degradation of humic acid-like and fulvic acid-like components explained an additional 10%. Interestingly, while humic-like components (E1–3 and 6) were not strongly excreted (Figure 2), they typically increased in fluorescence intensity during biodegradation, which suggests that they may also form as by-products of excreta degradation (Figure 4, PC2C). In most taxa, some fulvic acid-like components were bioavailable (E7 and 10) while others (E4) appeared to be by-products of degradation. Uniquely, in Tipula, E4 was consumed and E7 was produced—suggesting potential species-specific dynamics (Figure 4, PC2B).

3.4 | DOC uptake and comparison to insect excretion

From all six steady-state additions, the average DOC S_w was 184 ± a standard error of 33 m and average ambient uptake velocity was

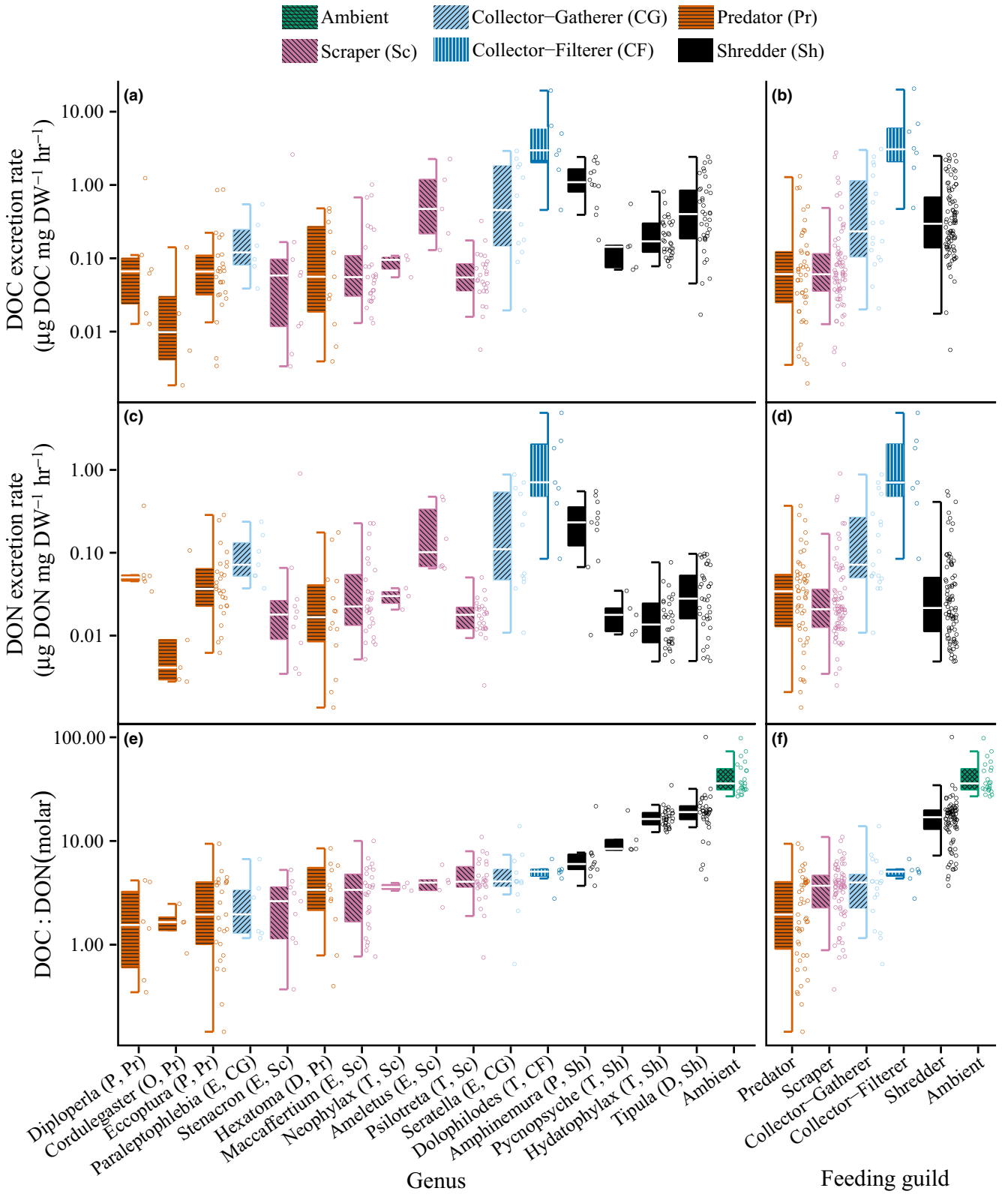

FIGURE 2 Excretion rates of dissolved organic carbon (DOC) by (a) genus and (b) feeding guild and dissolved organic nitrogen (DON) by (c) genus and (d) feeding guild. Stoichiometry of DOC:DON excretion by (e) genus and (f) feeding guild. Parenthetical letters after each genus consist of a one letter code for insect order Diptera (D), Ephemeroptera (E), Odonata (O), Plecoptera (P) and Trichoptera (T) and a two letter code for functional feeding group (see figure legend). All data are arranged in order of increasing median DOC:DON. Taxa with fewer than three observations have been removed. See Supporting Information Table S5 for all taxa

TABLE 1 ANCOVA testing for effects of body size, functional feeding group (FFG) and taxonomic identity on mass-specific elemental excretion rates and composition

Response	Variable	*df*	*F*	*p*	R^2_{adj}
DOC rate	Body size	1	50.7	<0.005	0.55
	FFG	4	42.9	<0.005	
	Taxon	19	4.5	<0.005	
DON rate	Body size	1	165.7	<0.005	0.57
	FFG	4	13.6	<0.005	
	Taxon	19	5.6	<0.005	
DOC:DON	Body size	1	48.1	<0.005	0.52
	FFG	4	48.7	<0.005	
	Taxon	19	1.7	0.038	

0.27 ± 0.04 mm/min. To provide a more conservative estimate of areal uptake, we used the slightly higher ambient BDOC estimate of 19.3% and calculated the areal uptake for labile C as 3.97 ± 0.67 mg labile DOC m^{-2} hr^{-1} (Table 2). Using an average areal biomass of insects of 1.76 g DW m^{-2} (Supporting Information Table S2) and the calculated community excretion rate, we estimate that insects released 2.31 mg DOC m^{-2} hr^{-1}.

4 | DISCUSSION

Digestively transformed DOM excreted by aquatic insects may be a significant resource for microbial heterotrophs in aquatic ecosystems, and a largely unmeasured flux in the biogeochemical cycles of C and N. Collectively, the quantity and quality of DOM excreted by insects are strongly influenced by their functional community composition, likely due to differences in diet (functional feeding group), with body size (metabolic rates) and taxon also exerting influences. Importantly, we observed DON excretion rates, while variable (Supporting Information Table S5), were almost double that of NH_4^+ excretion, suggesting that insects may release as much or more N in organic forms as compared to inorganic forms. When compared to ambient water column DOM, the high quality (low DOC:DON ratio) and greater bioavailability of excreted DOM indicate that aquatic insects may produce an important organic nutrient and energy resource for microbes. The release of labile DOC and DON by animals may be an important control or buffer in the competition between primary producers and microbes for limiting resources, as well as a feedback supporting microbial predator biomass. For biogeochemical cycles, our results suggest that if animal contributions to N cycling are only estimated using NH_4^+ excretion rates—common in animal-mediated elemental cycling studies—they may underestimate the true contribution to bioavailable N cycling (a 66% underestimate in the case of insects). Collectively, our data indicate the release of organic nutrients and energy by animals may be an important biogeochemical flux in streams that links the flow of energy and elements between green and brown food webs.

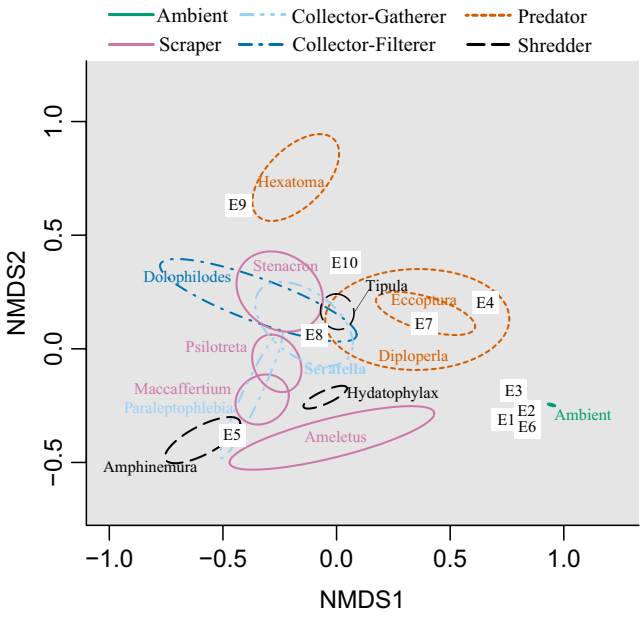

FIGURE 3 Non-metric multidimensional scaling (NMDS) ordination of the fluorescent dissolved organic matter (DOM) excretion composition and ambient stream water. Confidence (95%) ellipses were calculated for the mean of each genus for which more than five incubations were conducted. Ellipses are coloured by functional feeding group (FFG)

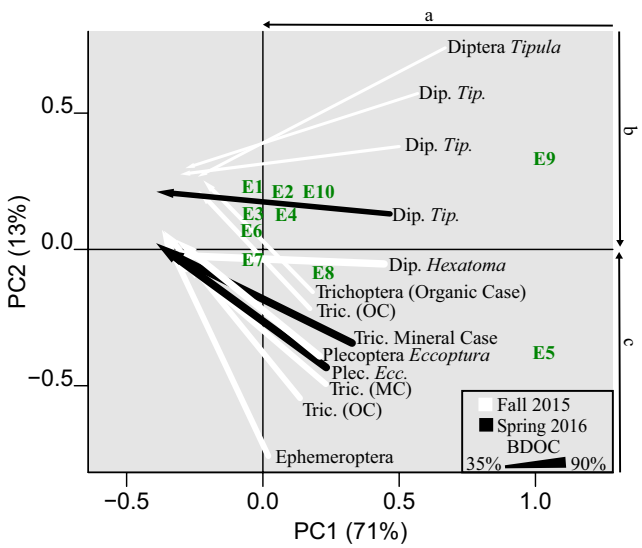

FIGURE 4 Principal components analysis of the bioavailability of dissolved organic carbon (BDOC) fluorescence composition of degradable components. The percentage of variance explained by each axis is indicated in parentheses. Location of arrow tails indicates the initial excreta composition, and arrowheads indicate the composition after 28 days of microbial degradation. Arrow size indicates the percentage of excreted dissolved organic carbon (DOC) that was degraded. Along PC1, (a) reflects the biodegradation of highly labile protein-like DOM. Along PC2, (b) reflects the degradation of excreta derived from vascular plant precursors produced primarily by *Tipula* (obligate shredder), and (c) reflects the degradation of protein-like components released by predators and omnivorous collectors and scrapers

TABLE 2 Spiralling metrics measured in the study watershed using steady-state addition. Ambient uptake U_{BDOC} for single compound is calculated according to Equation 3, and leaf leachates are corrected after Mineau et al (2016) (Supporting Information Appendix S1). For dissolved organic matter (DOM) additions on the same days in this study, order of addition is listed as 1 (first) or 2 (second)

Date	Discharge (L/s)	Ambient DOC (mg/L)	DOM added	Elements	Order added	S_w (m)	v_f (mm/min)	U_{BDOC}[a] (mg m^{-2} hr^{-1})	Reference
24/9/2015	0.5	1.10	Acetate	C	1	140.5	0.58	4.0	This study
24/9/2015	0.5	1.04	Citrate	C	2	250.4	0.33	2.1	This study
7/10/2015	0.5	1.51	Citrate	C	1	315.8	0.30	2.4	This study
7/10/2015	0.5	1.59	Acetate	C	2	134.9	0.71	5.9	This study
15/10/2015	0.5	1.21	Glucose	C	1	98.7	1.22	5.9	This study
15/10/2015	0.5	1.20	Pyruvate	C	2	165.2	0.73	3.5	This study
1999	8.7–20.2	0.6–0.9	Urea	C, N		129.9	1.65	2.5	1
1999	8.7–20.2	0.6–0.9	Glutamic acid	C, N		153.8	1.39	9.2	1
08/04/2009	30.0	0.31	Acetate	C		769.2	0.60	5.4	2
Autumn 2002	15.0	1.48	Tulip poplar labile	C, N		238.0	1.22	2.6	3
Autumn 2002	15.0	1.48	Tulip poplar semi-labile	C, N		4,546.0	0.07	0.4	3
Autumn 1979[c]	1.3	1.30	Sugar maple	C, N		48.0	1.13	20.5	4
Autumn 1979[c]	1.0	1.22	Spruce	C, N		71.0	0.70	12.1	4
Autumn 1979[b]	1.0	10.03	Sugar maple	C, N		73.0	0.61	87.4	4
Autumn 1979[b]	1.5	7.46	Spruce	C, N		154.0	0.50	53.3	4
July 1999[c]	0.5	1.10	Acetate	C		14	1.52	20.0	4
Autumn 2000[c]	0.6	2.01	Sugar maple	C, N		15	1.75	50.3	4
Autumn 2000[c]	0.8	2.01	Spruce	C, N		24	1.44	41.4	4
Autumn 2000[b]	2.0	5.99	Sugar maple	C, N		>500	0	0.0	4

[a]For this study, the highest measured background BDOC of 19.3% is used. For other studies employing simple compounds, to provide a conservative estimate, we assume a high background BDOC of 20%. [b]Cascade Brook. [c]Bear Brook. [1]Brookshire, Valett, Thomas, and Webster (2005); [2]Lutz, Bernhardt, Roberts, Cory, and Mulholland (2012), x4 background addition; [3]Kaplan, Wiegner, Newbold, Ostrom, and Gandhi (2008); [4]Bernhardt and McDowell (2008).

4.1 | DOM excretion rates from individuals to ecosystems

At the individual scale, DOC and DON excretions are regulated by organismal processes similar to those regulating inorganic nutrient excretion. Body size, taxonomy and diet represent the basic thermodynamic (metabolic) and stoichiometric controls regulating the intake and release of elements (Allgeier et al., 2015; Vanni & McIntyre, 2016). In our study, the pattern of decreasing mass-specific organic matter excretion rate with increasing body size parallels patterns reported for inorganic nutrients across taxa (Meyer & O'Hop, 1983; Vanni & McIntyre, 2016). Thus, environmental factors selecting for communities consisting of larger or smaller individuals (Chown & Gaston, 2010) may result in a lower or higher, respectively, net community excretion rate. Additionally, our results highlight the importance of functional community composition (feeding guild) and taxon on the flux and stoichiometry of animal-derived elemental cycling. Hence, factors controlling taxonomic and functional community assembly in streams should also affect the characteristics of DOM excretion at the community level. For example, increases in biomass of relatively higher excreting (small-bodied) collector-filterers with stream size (Benke, 1993) suggest that, per gram, insect communities in larger rivers may generate more DOM.

4.2 | Composition of DOM excretion

We documented taxon-specific differences in the composition of excreted DOM. The patterns in DOC:DON we observed likely represent a feeding continuum from shredders who are consuming mostly vascular plant tissues with high C:N ratios, to collectors and scrapers who are consuming detritus and increasing proportions of microbes and algae, to predators who are consuming other animals (Figure 2f) with a lower C:*N* (~5, Evans-White et al., 2005). These patterns were further supported by the distinct DOM fluorescence signatures of plecopteran predators (higher in fulvic acid-like fluorescence) and a dipteran predator (higher tyrosine-like fluorescence) compared with shredders, collectors and scrapers (Figure 3). For example, for the predator *Hexatoma*, high tyrosine excretion may be due to the excretion of unassimilated prey haemolymph, which is rich in tyrosine (Chapman, Simpson, & Douglas, 2013). Because many scrapers and collector-gatherers are omnivorous (Cross et al., 2007), the similar

fluorescence signatures (Figure 3) and DOC:DON ratios (Figure 2f) most likely reflect their shared resource base.

4.3 | Seasonal influences

Seasonal changes in temperature may produce seasonal patterns in macroinvertebrate DOM production. Metabolic rates of insects should roughly double with each 10°C increase in temperature (Reinhold, 1999). Comparing our data with published work examining the influence of land use on DOC excretion rates in Ephemeroptera (James et al., 2007), our average excretion rate for Ephemeroptera genera at ~11°C (1.038 μg DOC·mg DW^{-1} hr^{-1}) was ~33% of the rates described in James et al. (2007; ~3 μg DOC·mg DW^{-1} hr^{-1}), which were likely collected at 19–25°C (Di Rocco, Jones, & Chu, 2015). These data suggest insect communities may release relatively more DOC per gram insect biomass during warmer seasons.

As many stream insects are omnivorous (Cross et al., 2007), increased primary production in response to enhanced light availability may result in animals shifting from brown to green food web resources (Collins et al., 2016). Light availability in streams changes seasonally and with longitudinal position in a river network (Vannote, Minshall, Cummins, Sedell, & Cushing, 1980). Thus, when insolation and/or temperatures reach seasonal lows, microbial production may be more strongly supported by animal-mediated organic matter transformations in the brown food web. Conversely, when insolation and temperatures are higher, microbial production may increase dependence on resources derived from animal-mediated organic matter transformation in the green food web.

In our site, however, a shift from brown to green food web resources was not a likely explanation for the seasonal differences we observed in excretion quality since primary production did not appear to exert any diel influence on oxygen concentrations regardless of light availability (Supporting Information Figure S3). The lack of relationship between oxygen concentration and light availability in our site may have been due to shading by the tree canopy and the terrain, and/or the low ambient nutrient concentrations we observed throughout the study period. The seasonal changes in excretion quality we documented may have been due to temporal variation in the quality of organic matter. Specifically, we observed ~1.5 times higher BDOC in the DOM excreted by non-predators (shredders, collectors, scrapers) in the autumn compared to the spring. This may be due to more rapid gut passage times and lower assimilation efficiencies when food resources are abundant but "low quality" (high C:N), as is the case with fresh leaf litter in the fall. However, when food is less abundant, but higher quality after microbial conditioning, as may be the case in the spring, the same animals may exhibit higher assimilation efficiencies and longer passage times (DeMott, McKinney, & Tessier, 2010). In comparison, the BDOC of DOM excreted by predators—whose insect prey composition and availability probably exhibit less seasonal variability—was similar in both seasons.

4.4 | Fate of excreted DOM: linking brown and green food webs

Our data suggest that animal-mediated transformation of ingested organic matter (detritus, prey, primary producers) provides a significant flux of labile energy (DOC) and nutrients (DON) to heterotrophic microbes. Recent biogeochemical models describe the coupled dynamics between organic matter resources and microbial communities by separating organic matter pools based on their chemical composition or biological reactivity (e.g., Buchkowski, Bradford, Grandy, Schmitz, & Wieder, 2017; Guenet, Danger, Abbadie, & Lacroix, 2010). While conceptual and numerical food web models capture animal waste fluxes as sources of detritus and nutrients (particulate organic matter; e.g., Carpenter, Cole, Pace, & Wilkinson, 2016; Moore et al., 2004; Zou et al., 2016), few models represent the production of labile dissolved organic nutrients and energy, which are readily available to heterotrophic microbes. Thus, we propose two extensions to enhance food web models to formalize biogeochemical dynamics in the flow of organic nutrients and energy between green and brown food webs (Figure 5).

First, the inclusion of a labile organic energy pool and flux (DOC, Figure 5a) would allow for the explicit inclusion of a positive DOC transformation feedback between animals and microbes (Figure 5a–c). While this feedback from animals to microbes (Figure 5a,b) is explicit in some models ("incomplete digestion"—Jumars et al., 1989; "recycling"—Nagata, 2000; "ontogeny"—Moore et al., 2004) and may be significant in some marine models (e.g., Nagata, 2000: satisfying 42.5% of food web C demand), it is deemphasized in lotic ecosystems (Meyer, 1994). In our small stream ecosystem, release of organic energy by insects could, conservatively, satisfy 40% ± 7% of the estimated 3.97 mg m^{-2} hr^{-1} ambient demand for labile DOC (assuming an average DOC bioavailability of 70% or 1.61 mg BDOC m^{-2} hr^{-1}). Though limited, research has documented the subsequent feedback between insect released-microbially assimilated labile C and insect production (Figure 5a–c; Ings, Hildrew, & Grey, 2010).

Second, integrating a labile organic nutrient pool (Figure 5d) produced by all food web members (plants, microbes, and animals), but only available for microbial uptake (Figure 5e), would enhance existing food web models by buffering competition between plants and microbes for inorganic nutrients. In the case of DON, our work suggests the proportion of bioavailable organic nitrogen exceeds the proportion of inorganic N released (~2:1 total, 1.4:1 bioavailable) and may constitute a relatively larger pool for bacterial uptake. In marine systems, dissolved free amino acids are tightly cycled sources of N for microbes with uptake rates exceeding those of nitrate and comparable to ammonium (Keil & Kirchman, 1991). While we did not measure DON uptake in the field, excreta contained fluorescence signatures of several amino acids and consistently high levels of bioavailable DON across taxa. The high bioavailability and amino acid nature of this excreted DON may be critical for maintaining microbial C-limitation/colimitation (DOC:DON excreted = 4.6:1, microbial biomass ~7:1) and

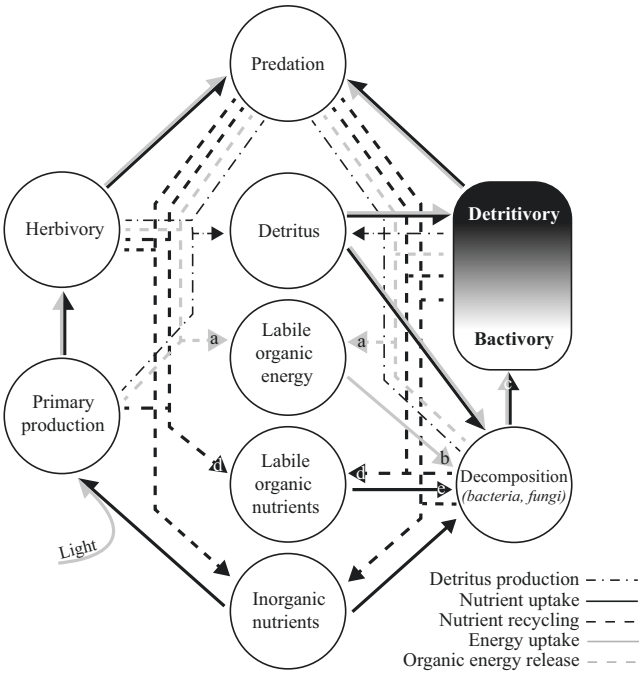

FIGURE 5 Conceptual model highlighting the labile organic nutrients and labile organic energy, in addition to inorganic nutrients, as critical nexuses of interaction between brown and green food webs. All arrows represent elemental mass fluxes (production or utilization of elemental mass per unit time), and circles represent pools or standing stocks (mass) of: labile organic energy production (a) and uptake (b); microbial decomposer biomass uptake (c); labile organic nutrient production (d) and uptake (e). While graphically depicted as separate pools, they are physically and numerically linked. For example: *Physically*, molecules with DOC may exist independently of DON; however, molecules containing DON must always contain carbon (DOC). In this way, microbes may utilize DON as both an energy and a nutrient source depending whether C or N is limiting (Cherif & Loreau, 2013; Lutz, Bernhardt, Roberts, & Mulholland, 2011). *Numerically*, DON production or utilization, nominally, is a function of detritus ingestion rate and elemental imbalance between animal and food resource (Cherif & Loreau, 2013). The gradient box indicates the gradient in diet (omnivory) from bactivory to detritivory of animals in the brown food web. In stream ecosystems, this gradient may be better represented by a ternary gradient between herbivory, detritivory and bactivory (Collins et al., 2016; Hall & Meyer, 1998)

reducing competition for inorganic resources between primary producers and microbes (Daufresne et al., 2008).

Our research highlights the potentially important role animals play in mediating the regeneration of bioavailable organic nutrients and energy from organic food resources. The regeneration of organic nutrients and energy creates a trophic feedback between animals and microbes that links green and brown food webs beyond inorganic nutrients. Understanding and quantifying the magnitude of such linkages is essential to predicting how ecosystems may respond to anthropogenic perturbations. Human disturbances altering the biomass, community composition, body size and resource stoichiometry of animal communities in either food web may cascade through both. Microbial access to abundant animal-derived organic nutrients that are less available to primary producers may help buffer cascades from green to brown food webs but transmit cascades from brown to green food webs. Integrating these animal-mediated transformations of organic matter more effectively into biogeochemical cycling and food web models will allow us to better understand how and when animal community composition and functions shape element cycling and ecosystem persistence.

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AUTHOR CONTRIBUTIONS

All authors contributed to the design and conceptualization of the experiment and contributed substantially to revision of the first draft of manuscript; T.B.P. and K.A.M. collected the data; and T.B.P. prepared and wrote the first draft of manuscript.

DATA ACCESSIBILITY

Data are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.ts8fd6m (Parr, Metcalf, Inamdar, & Capps, 2018).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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