DEATH AND DECOMPOSITION IN AQUATIC ECOSYSTEMS

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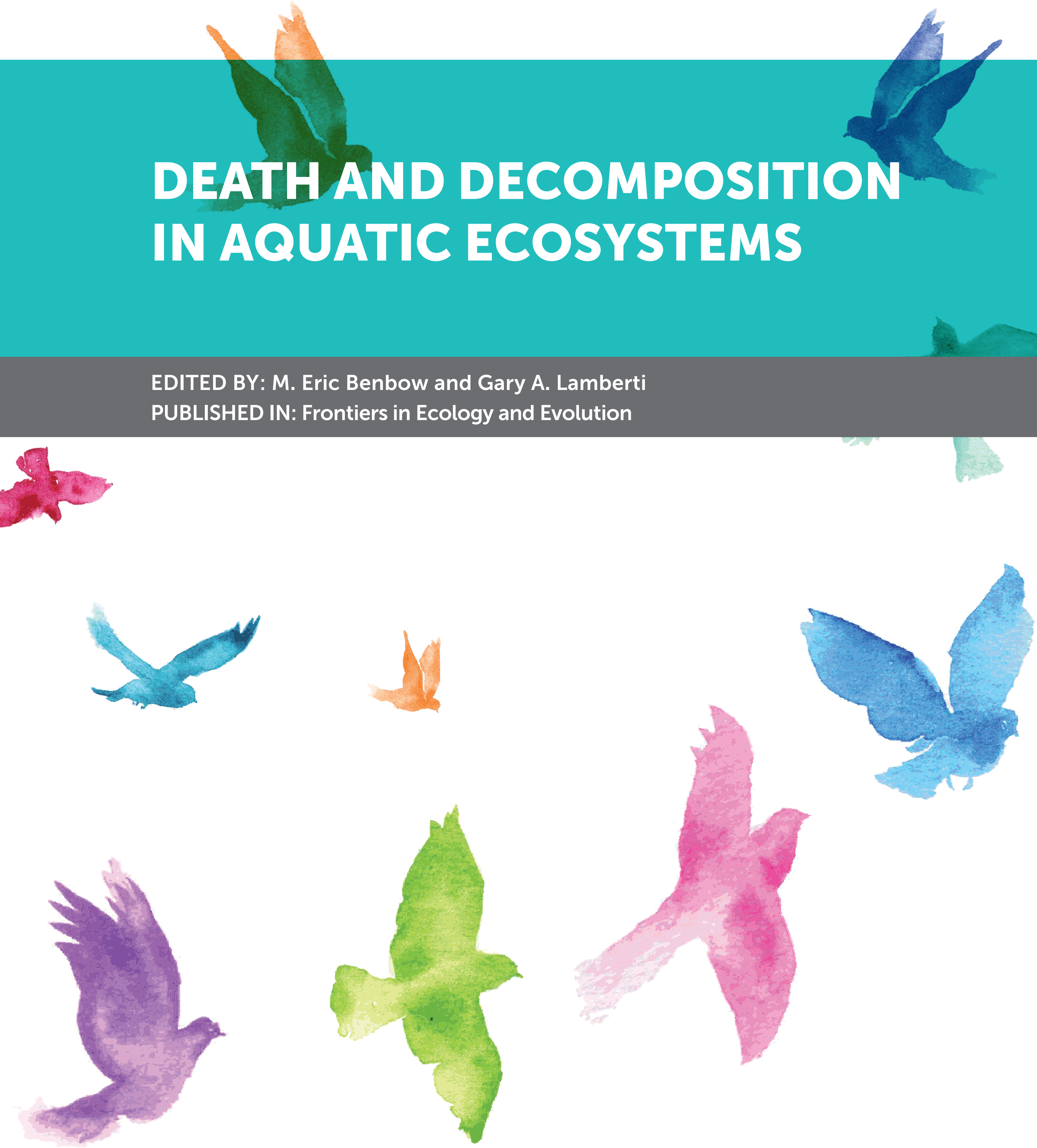

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DEATH AND DECOMPOSITION IN AQUATIC ECOSYSTEMS

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Death and Decomposition in Aquatic Ecosystems

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Resource subsidies affect nutrient cycling, species interactions, and food webs in ways that influence ecosystem structure and function, but their effects depend on the history, magnitude, and recurrence frequency of the subsidies. In aquatic ecosystems, plant detritus has been considered the predominant form of such subsidies; however, while considered less abundant in many ecosystems, carrion represents subsidies with relatively rapid turnover and highly concentrated nutrient and energy release that can have strong and lasting effects on ecosystems. Carrion subsidies can be both autochthonous or allochthonous, and come in the form of natural senescence or disease-related non-consumptive mortality, phenology-based programed death (e.g., salmon spawning and death), or stochastic and episodic events (e.g., mass fish die-offs). All aquatic ecosystems have some level of non-consumptive mortality that provides a background level of carcasses to aquatic ecosystems, while others have a natural history of carrion resource subsidies (e.g., natural salmon-bearing streams), and some have only recently been exposed to phenology-based carrion subsidies (e.g., anthropogenic salmon introductions around the world). Many aquatic ecosystems experience episodic subsidies in the form of unexpected mass mortalities (e.g., eutrophication-, disease-, or climate-related mass die-offs) or have seasonally dependent pulses, like that of marine or lake snow in the form of zooplankton carcasses. The responses of ecosystems to these different histories and frequencies of carrion subsidies have often been independently investigated, with little effort to compare and bridge research boundaries in the broader context of resource subsidies. In this review, we provide a synthesis of how pulsed carrion nutrient and energy subsidies have widespread and lasting impacts on many aquatic ecosystems. We do this with a synthesis of literature from freshwater and marine ecosystems along three themes of how carrion is produced and decomposes: autochthonous and allochthonous necromass; phenology-based mortality; and stochastic and episodic mass mortality subsidies. Studies of charismatic megafauna carrion (e.g., whales) have described significant impacts in deep ocean systems, but much less is understood for other groups of animals. Quantifying the energy, nutrient, and foodweb effects of carrion is needed for more species and among habitats to more fully understand how ubiquitous forms of necromass contribute to aquatic ecosystem structure and function.

Keywords: decomposition, microbial, ecosystem metabolism, community assembly, necrobiome, carrion, mass mortality, forensics

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INTRODUCTION TO NECROMASS RESOURCE SUBSIDIES IN AQUATIC ECOSYSTEMS

Background

Decaying organic matter, or necromass, comes in the form of both plant and animal biomass, recently differentiated as autotrophically (e.g., leaf litter) and heterotrophically (e.g., carrion) derived biomass, respectively, and is recycled back into ecosystems by the necrobiome community (Benbow et al., 2019). While both forms of necromass are considered important to ecosystems, plant detritus processing has historically dominated research as the main energy pathway in aquatic ecosystems (Vannote et al., 1980; Webster and Benfield, 1986; Getz, 2011; Boyero et al., 2016), especially for lotic systems lacking anadromous fish populations (Moore et al., 2004; Gessner et al., 2010; Benbow et al., 2019). Until recently (McDowell et al., 2017; Subalusky et al., 2017; DuBose et al., 2019; Wenger et al., 2019), aquatic studies of animal necromass (carrion) have largely focused on fish carcass decomposition (Richey et al., 1975; Garman, 1992; Schindler, 1992), or lake/marine snow represented by zooplankton carcasses in the water column of lentic ecosystems (Alldredge and Silver, 1988; Grossart and Simon, 1998; Giering et al., 2014). Some research has addressed the long-term decomposition of whale (and other vertebrate) falls in the oceanic abyss (Allison et al., 1991; Bennett et al., 1994; Smith and Baco, 2003; Kemp et al., 2006; Higgs et al., 2014). Much of past research on carrion in aquatic ecosystems has focused on species that are large in size, have anthropogenic importance (e.g., salmon runs), or elicit public intrigue (e.g., whale falls); however, a large portion of aquatic ecosystem necromass comes in the form of smaller organisms (e.g., phytoplankton, zooplankton, and invertebrates) with fast generation times and high turnover rates that substantially contribute to ecosystem production (Waters, 1977; Benke, 1998; Huryn and Wallace, 2000; Landry and Calbet, 2004; Patrick et al., 2019). While we acknowledge the importance of phototrophically derived necromass in aquatic ecosystems and the associated trophic relationships (Little and Altermatt, 2018), we direct interested readers to the many extensive reviews and empirical research of such resources ranging from phytoplankton, seaweed, and macrophytes to large wood debris (Lindeman, 1942; Mann, 1969; Wallace et al., 1999; Moore et al., 2004; Entrekin et al., 2009; Tank et al., 2010).

For this review, we focus on heterotrophically derived necromass (from bacteria to whales) with emphasis on animal carrion, and also acknowledge that dung, frass, and other forms of animal tissue (e.g., gametes) contribute to the larger resource pool (Subalusky et al., 2015; Dutton et al., 2018; Subalusky and Post, 2018; Benbow et al., 2019). For an informative argument for the importance of egested heterotrophically derived forms of particulate organic matter to energy budgets of pelagic zones of lake ecosystems we direct readers to Wetzel (1995).

In general, the importance of carrion has been qualitatively considered relatively greater in large lakes and oceans (Alldredge and Silver, 1988; Smith and Baco, 2003; Tang et al., 2014) compared to shallow wetlands and marshes (Brinson et al., 1981; Duggins et al., 1989). However, Subalusky and Post (2018) discuss how recipient ecosystem qualities (e.g., productivity or trophic structure) are important for understanding cross-system effects of carrion. In watersheds, carrion has been largely studied as pulsed resource subsidies like fish die-offs (Parmenter and Lamarra, 1991) or as part of programed phenologically driven semelparous death like salmon life cycles (Moore et al., 2004; Benbow et al., 2019). Regardless of origin, most organic matter ultimately decomposes and is recycled in ecosystems (Wetzel, 1995; Moore et al., 2004; Benbow et al., 2019), providing an intimate connection between ecosystem structure and function through interacting necrobiome species responsible for the breakdown (physical destruction into smaller and smaller units) and decomposition (biochemical alteration and conversion) of necromass (Lindeman, 1942; Putman, 1983). This process of decomposition, or turnover rate, is also known to be much faster for carrion compared to plant litter biomass, and is more important to ecosystems than once considered (Barton et al., 2019).

Precedent From Terrestrial Ecosystems

Most studies of carrion ecology have been conducted in terrestrial ecosystems. Here we provide examples from terrestrial habitats to show the potential of carrion to ecosystems, which can potentially be transferred to aquatic conditions. In terrestrial systems, carrion has often been thought to contribute marginally to ecosystem energetics (Swift et al., 1979; Barton et al., 2019). However, Barton et al. (2019) argue that because of the high turnover rate of carrion, carcasses have historically gone understudied since they are quickly recycled back into the ecosystem and are hidden from observation; thus, they have been presumed to have a negligible contribution to energy and nutrient flow in ecosystems. This view is plausible because it has been historically difficult to quantify natural rates of carrion decomposition in ecosystems, and so the relative production of energy from carrion compared to the production from an equivalent amount of plant necromass may be disproportionate and underestimated (Barton et al., 2019). As one example (see review by Scott, 1998), burying beetles have been shown to remove and conceal 91% of exposed small (21–210 g) mammal carcasses by burial within an average of 1.4 days (Trumbo, 1992). In the same study, 22.7% of the exposed carcasses were eaten or removed by vertebrate scavengers. This example demonstrates how quickly small carrion can be removed from scientists' observation.

Decomposing carcasses contribute hot spots of nutrient release (Carter et al., 2007; Benbow et al., 2019) that in terrestrial systems can have direct and indirect long-term (e.g., months to years) effects on soil conditions (Bornemissza, 1957; Strickland and Wickings, 2015), plant communities (Towne, 2000; Wardle et al., 2004), and both invertebrate and vertebrate scavengers (Bump et al., 2009; Beasley et al., 2012; Barton et al., 2013; Benbow et al., 2015). The spatial extent of such effects in ecosystems has not been well documented, but could be potentially important over decades (Bump et al., 2009). As an example of this potential, Hawlena et al. (2012) found that fear of spider predation by living grasshoppers resulted in carcasses with significantly higher carbon to nitrogen ratios than non-stressed specimens, and the resulting change in carcass quality, even when the biomass was about 140 times lower than the plant litter biomass, affected below ground community function and subsequent leaf litter decomposition on carcass sites in an old prairie ecosystem. Thus, even small amounts of carrion biomass have significant effects on measurable ecosystem processes, such as leaf litter decomposition and soil function, suggesting that if scaled by population density, mortality rates, and turnover in a landscape carcasses may significantly impact ecosystem functions in ways yet to be examined. If scaled in this manner, carrion will likely be shown to have collective ecosystem level effects like those recognized for ungulate dung and urine deposition in prairies (Norman and Green, 1958; Seastedt et al., 1991) or hippopotamus dung for some African rivers (Subalusky et al., 2015).

The Need to Scale From Carcass to Collective Effects

The hypothesis that individual carcass effects can have large spatial and long temporal scale effects has been supported by work in Isle Royale National Park, Michigan (Bump et al., 2009). In this 50-year study of the effects of over 3,600 wolf-killed moose carcasses on landscape heterogeneity and ecosystem function, Bump et al. (2009) reported that soil nutrients (e.g., 100–600% higher inorganic nitrogen at carcass compared to control sites), microbial biomass, microbial community composition, and surrounding plant leaf nitrogen (e.g., leaf nitrogen was 25–47% greater at carcass sites) was significantly elevated for at least 2–3 years at carcass deposition locations. In case studies of whale falls, Smith and Baco (2003) estimate that the sediment beneath whale carcasses receive a pulse of organic matter that is equal to almost 2000 years of background material over decomposition. Furthermore, in observations on large elasmobranch (i.e., whale shark and mobulid rays) carcass falls, Higgs et al. (2014) estimated that such carcasses represent on average about 4% of the normal particulate organic carbon flux to the seafloor in the bounding area of their occurrence. The authors suggest that the deep-sea scavenger communities benefit most from these significant energetic subsidies.

The same considerations for the underappreciated role of carrion in terrestrial ecosystems or the deep sea abyss is true for other aquatic ecosystems, where studies have documented localized carcass effects in salmon-bearing streams, whale falls, and other fauna of the deep ocean benthos (Smith and Baco, 2003; Kemp et al., 2006; Anderson and Bell, 2014; Higgs et al., 2014), including prehistoric carrion (Reisdorf et al., 2012; Danise et al., 2014); fish and waterfowl carcasses of salt marshes (Parmenter and Lamarra, 1991); and additional carcass effects likely in most aquatic habitats.

Indeed, vertebrate carrion placed in deep (300–3000 m) marine habitats initiates a succession of both invertebrate and vertebrate scavengers that take advantage of this punctuated, heterotrophically derived subsidy (Kemp et al., 2006; Anderson and Bell, 2016). What is less understood is how individual carcasses scale with mortality at population and community levels of biological organization. New studies are needed to better use population demographics and mortality estimates for calculating ecosystem level carrion production from species that do not have phenology-based or episodic mass mortalities. In Figure 1 of Barton et al. (2019) aquatic carrion biomass ranged from 10^2 kg/km^2 for copepods up to 10^7 kg/km^2 for bivalves, with different species of fish ranging from about 10^3 to 10^6 kg/km^2. Among the examples they provided, terrestrial vertebrate biomass was at least two orders of magnitude lower than aquatic species at 10^3 kg/km^2. While this example is not comprehensive, it does suggest that the effect of heterotrophically derived necromass in ecosystems is likely much greater than historically considered. To better incorporate the contributions of carrion in aquatic ecosystem energy and nutrient budgets, additional studies are needed that directly quantify carcass necromass and turnover across biomes. Such broadly available data on non-consumptive heterotrophically derived necromass in ecosystems would advance theory in consumer-resource and food web ecology (Getz, 2011).

In this paper we support the arguments of Subalusky and Post (2018) and Barton et al. (2019) that carrion resources are important subsidies in most ecosystems. We do this with a synthesis of literature from freshwater and marine ecosystems along three themes of how carrion is produced and decomposes through the following: autochthonous and allochthonous necromass decomposition; phenology-based mortality; and stochastic and episodic mass mortality subsidies. We provide examples and case studies that collectively show that carrion, along a size continuum that spans multiple orders of magnitude, likely plays an underappreciated role in ecosystem energetics and nutrient dynamics. Furthermore, we posit that death links living resource pools of multiple and interacting populations of species through pathways of non-consumptive mortality that result in a dead resource pool represented by carcasses that are quickly decomposed and recycled back into the living resource pool through detritivores and omnivores (**Figure 1**). In aquatic systems, such carrion represents autochthonous or allochthonous energy and nutrient subsidies that affect foodwebs and ecosystem function in complex and understudied ways.

AUTOCHTHONOUS AND ALLOCHTHONOUS CARRION NECROMASS

Similar to phototrophically derived detritus, carrion necromass in aquatic ecosystems can come in the form of autochthonous and allochthonous sources. Carrion necromass of aquatic ecosystems can be generated from within or outside of a system in the form of autochthonous and allochthonous resources, respectively (**Figure 1**). As part of the autochthonous resource pool, carcasses resulting from natural senescence, physiological intolerance, or disease-related death of aquatic heterotrophs enter the detrital pool based on rates of mortality, disease, partial predation, and generation times related to natural senescence. These resources

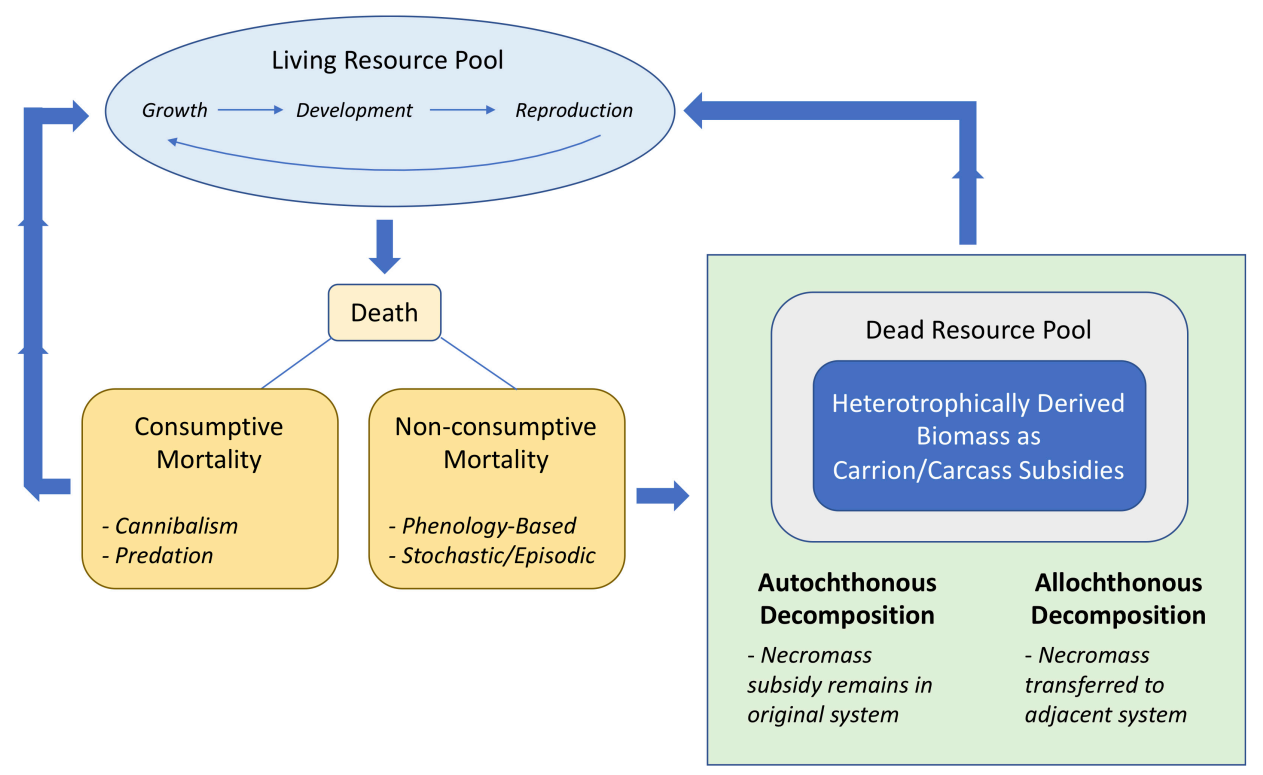

FIGURE 1 | Conceptual diagram linking living resource pools to dead resource pools through the death and decomposition of heterotrophically derived biomass in the form of carrion subsidies. Each species is part of a living resource pool that encompasses growth, development, and reproduction; however, death of a proportion of the living individuals leads to the scavenging or decomposition of the once living biomass (or necromass) that ultimately becomes incorporated into the living resource pool through food web interactions. Death can come in the form of consumptive mortality (i.e., cannibalism or predation) where the nutrients and energy of dead organisms goes back to the living resource pool. Death can also come in the form of non-consumptive mortality, as part of natural senescence of the life cycle that can be through phenology-based (e.g., salmon life cycle) or stochastic/episodic (e.g., mass mortality of bivalve populations) processes. The resulting decomposition of these carrion subsidies can be autochthonous or allochthonous, depending on the life history and range of the once living species, affecting energy and nutrient flow within and among ecosystems.

represent necromass from all organisms that live in aquatic habitats, from bacteria to blue whales (Minshall et al., 1991; Fenoglio et al., 2005; Subalusky and Post, 2018).

For any specific taxon, the degree of natural senescence, physiological intolerance (e.g., temperature, physical and chemical thresholds), or disease-related non-consumptive death contribute to the within-system detrital pool and associated energy and nutrient dynamics. For instance, non-consumptive mortality is a natural part of the population dynamics of any species, but the overall magnitude is rarely quantified, except for studies related to aquaculture production of commercially important taxa (Rowe et al., 1989; Karunasagar et al., 1994; Chen et al., 1995; Lorenzen, 1996). Furthermore, the mere presence of predators is known to have negative effects on life history traits and fitness of many species (Preisser et al., 2005). Since many organisms are prey for a variety of predators, this collective effect of non-consumptive predation threats can lead to facilitated senescence within many animal populations, although this form of non-consumptive mortality has not been broadly studied. In general, non-consumptive predator effects have been documented to be as strong or stronger than direct predation effects for many species, and the non-consumptive predator presence effect is generally stronger in aquatic than terrestrial ecosystems (Preisser et al., 2005).

Non-consumptive effects can come in the form of increased costs of defensive strategies that include lower mating success, increased vulnerability to other predators, energetic investments related to finding resources or defensive structures (e.g., morphology or biochemical), or through reduced survivorship (Kotler et al., 1993; Preisser et al., 2005; Sheriff et al., 2009). For instance, McCauley et al. (2011) reported significant non-consumptive predator induced mortality and failed metamorphosis to adults in dragonfly larvae exposed to predatory fish and other dragonfly predators. Depending on predator density, survivorship was 1.2–4.3 higher under no-predator mesocosm conditions. The mechanisms responsible for such non-consumptive predator induced mortality are not clear, but have been speculated to be related to increased susceptibility to disease and energetic costs of foraging related to induced stress (Ramirez and Snyder, 2009; Hawlena and Schmitz, 2010). This example demonstrates that just the presence of a predator leads to greater mortality, supporting the hypothesis that there are degrees of non-consumptive mortality that occur in natural environments; however, the mechanisms and sources of non-consumptive mortality are difficult to identity and quantify. Such non-consumptive mortality may result in increased heterotrophically derived necromass contributions to ecosystem organic matter budgets through decomposition (**Figure 1**).

The non-consumptive component of population mortality has not been studied in any detail for most aquatic organisms, and is often presumed negligible in ecosystem level budgets or calculations of secondary production where predator consumption rates have often been assumed to lead to 100% prey mortality (Hynes, 1970; Waters, 1977; Wetzel, 1995). This absolute consumption rate is not often the case in natural systems, with many individuals of a population avoiding predation and succumbing to other means (e.g., disease or starvation) of mortality (Wetzel, 1995). Thus, the availability of carcasses to aquatic ecosystems is likely larger than previously assumed, especially if the effects of individual carcasses are scaled by their collective density and rates of availability in the environment as discussed by Barton et al. (2019).

The effect of individual carcasses on ecosystem structure and function has been shown to be variable (Minshall et al., 1991; Fenoglio et al., 2005; Barton et al., 2019; Benbow et al., 2019); however, individual carcass effects have not been scaled to account for population level mortality over space and time in a way that would reveal the collective pool of heterotrophically derived necromass for ecosystems based on natural, non-consumptive rates of mortality (Barton et al., 2019). A significant challenge to addressing this need lies with differentiating natural senescence, starvation, climate, or disease-related mortality from predation or consumption rates for a specific population. Quantifying non-consumptive mortality is inherently difficult, but could potentially be done by evaluating life tables (Deevey, 1947) of organisms with and without predators, much like that for humans (Haldane, 1953). Additional studies are needed to devise ways to account for non-consumptive mortality, much like that of recent examples that quantified seal (Quaggiotto et al., 2018) and wildebeest (Subalusky et al., 2017) mortality on aquatic ecosystems. However, there have been surprisingly few studies on non-consumptive mortality of micro- and macroinvertebrates; those taxa that can often have fast generation times and high mortality.

Potential ways to quantify non-consumptive mortality of macroinvertebrates can come from life history and secondary production studies in the absence of predators in natural ecosystems or from more artificial conditions, such as those conducted for ecotoxicology studies and aquaculture. Some estimates of macroinvertebrate secondary production are available from fish-bearing and fishless lakes (Arnott and Vanni, 1993; Northington et al., 2010), but the body size distributions are not often reported to allow for estimates of non-consumptive mortality and how that may mediate secondary production. It is often assumed in studies of secondary production that population loss can all be attributed to predation, but this is likely not the case. Data from control groups (e.g., no treatment with a contaminant) used in ecotoxicology studies may also provide data on non-consumptive mortality, but often these experiments are done under artificial conditions associated with laboratory or field mesocosms, where densities and abiotic conditions may not represent natural conditions (Rand et al., 1995; Boudou, 2018). These artificial conditions are similar to aquaculture systems and associated research (Huet et al., 1986). The degree to which non-consumptive mortality in aquaculture conditions differs from natural ecosystems is not well understood. Another potential mechanism for determining non-consumptive mortality in aquatic populations, and employed in fisheries management, is to derive mortality–weight relationships in populations in different natural ecosystems compared to conditions with eliminated or significantly reduced predation pressure (Lorenzen, 1996, 2000). For instance, Lorenzen (1996) reported allometric scaling of fish mortality to non-predatory mortality by modeling mortality–weight relationships of fish populations from natural ecosystems compared to ponds/cages and tanks with no predation pressure. In these conditions, mortality is attributed to diseases, water quality problems, or winter starvation (Huet et al., 1986). The derived weight exponents of mortality were consistently negative for populations in ponds/cages/tanks compared to natural ecosystems, suggesting non-predatory mortality is more weight (i.e., surrogate for age) dependent than is predatory mortality.

Additional information on carrion impacts on aquatic ecosystems can be gathered from mass mortality studies of autochthonous heterotrophs (see the section "Stochastic and Episodic Mass Mortality and Decomposition") and those related to programed phenology-based death of allochthonous taxa. As part of allochthonous resources, carcasses ultimately come from outside of the system and may include anadromous (e.g., salmon, sturgeon) or catadromous (e.g., eels) vertebrates, crustaceans, and molluscs (e.g., amphidromous shrimp and snails) that spend a portion of their life cycle growing and developing in other habitats or ecosystems (e.g., ocean or mangroves), but complete their life cycle in the freshwater environment (McDowall, 1988; Cederholm et al., 1989; Thuesen et al., 2011; Weaver et al., 2018). These resource subsidies can be made available through natural senescence, physiological intolerance, starvation, or disease-related, non-consumptive death (e.g., amphidromous herring) or through programed phenology-based mortality (e.g., post-spawning salmon). The effects of allochthonous sources of necromass have been studied in considerable detail, especially for salmon, both within its native range (Cederholm et al., 1989; Schindler, 1992; Chaloner et al., 2002; Janetski et al., 2009), but also where it has been introduced to naïve watersheds (Richey et al., 1975; Schuldt and Hershey, 1995; O'Toole et al., 2006).

Parasitized terrestrial insects can also represent allochthonous inputs in aquatic systems, entering streams from the riparian canopy due to modified behavior associated with their parasites (Schmidt-Rhaesa, 2001; Thomas et al., 2002). In one well-documented example, the horsehair worm (Nematomorpha) infects the camel cricket (Orthoptera: Rhaphidophoroidae) in Japanese watersheds. Once infected, the riparian crickets either slowly enter or jump into the streams, upon which the parasite leaves the body immediately or soon after the cricket is dead. These terrestrial subsidies have been shown to lower predation of resident aquatic invertebrates (Sato et al., 2008, 2011a,b, 2012). While not as well studied, other parasite-infected terrestrial insects (e.g., praying mantis) can also enter water with immediate horsehair release (Schmidt-Rhaesa, 2001). These examples suggest that parasitized insect subsidies enter the detrital pool upon death if not consumed, and may also offer a predatory release of other species that could potentially lead to additional non-consumptive mortality of those species in stream ecosystems.

PHENOLOGY-BASED MORTALITY AND DECOMPOSITION

A variety of aquatic organisms use phenology-based cues, such as temperature and day length, to control important behaviors including migration, hatching, and spawning. While these behaviors play critical roles in the fitness of the organisms themselves, they also affect other taxa throughout the food web. For example, the movement of anadromous fish can transport large amounts of nutrients and biomass upstream through their spawning activities and phenology-based mortality (Cederholm et al., 1999; Schindler et al., 2003; Wipfli et al., 2003), which are then used by other consumers (Bilby et al., 1996; Chaloner et al., 2002; Baxter et al., 2005; Hocking and Reynolds, 2011). Although mass mortality events of semelparous fish (e.g., salmon) following spawning are a dramatic introduction of necromass into freshwater systems, the release of eggs (which commonly exhibit high rates of mortality) and excrement by both iteroparous and semelparous fish comprise an important, though less studied, component of the available necromass, in some cases exceeding the nutrient inputs from carcasses (Tiegs et al., 2011; Childress and McIntyre, 2015). These inputs represent a major linkage of marine and freshwater systems as many anadromous fish derive most of their mass (>95%) from marine-based sources before migrating into freshwater systems via migration and subsequent semelparous death (Mathisen et al., 1988; Cederholm et al., 1999; Lamberti et al., 2010).

In contrast to stochastic mass mortality events, phenology-based events provide a regular input of nutrients into aquatic systems, with many organisms altering their life histories to coincide with these predictable influxes of resources (Hocking and Reynolds, 2011; Lisi and Schindler, 2011; Deacy et al., 2017). Predators, such as mink (*Mustela vison*) or brown bears (*Ursus arctos*), alter their behavior and timing of reproduction to coincide with the availability of salmon runs (Ben-David, 1997). These scavengers use salmon as a major nutritional source, in some locations obtaining >90% of their carbon and nitrogen from these fish (Willson and Halupka, 1995; Hilderbrand et al., 1996, 1999). The landscape can also affect how salmon carcass resource subsidies impact terrestrial and estuarine ecosystems, with differences reported in how wolves and bears transport carcasses to riparian forests and meadows depending on stream size and location within the watershed (Harding et al., 2019). A range of other mammals, birds, and insects use the carcasses of anadromous fish as resources that can affect the decomposition dynamics in streams (Cederholm et al., 1989; Zhang et al., 2003) and alter their behavior to better use and consume these resources (Moore and Schindler, 2010). In addition to direct consumption by eukaryotes, carcasses in aquatic systems can have strong interactions with microbial communities (Wipfli et al., 1998; Pechal and Benbow, 2016; Pechal et al., 2019). Although the impact of decomposing carcasses is highly dependent on biotic and abiotic factors (e.g., stream physical structure, riparian conditions, organisms present, etc.) the input of nutrients from carcasses stimulates microbial activity and primary production (Mitchell and Lamberti, 2005), leading to additional effects on higher trophic levels (Wipfli et al., 1998; Cederholm et al., 1999; Gende et al., 2002).

Salmon carcasses can also act as a resource subsidy to estuaries of salmon-bearing streams (Cederholm et al., 1999; Gende et al., 2004; Cak et al., 2008), with linkages to macroalgae through copepods (Fujiwara and Highsmith, 1997), marine invertebrates including echinoderms and crustaceans (Reimchen, 1994), and vertebrate taxa, such as coyotes and wolves (Gende et al., 2004). The availability of these resources to estuary systems can be mediated by complex interactions among trophic groups and habitat conditions. While the feeding activity of gray wolves (*Canis lupus*) and bears (*Ursus* spp.) can transfer carcasses from stream reaches to riparian habitats (Gende et al., 2004), where they become available to other scavengers, how many and which species of salmon they transfer depends on both species-specific interactions and landscape structure (e.g., riparian habitat and length of spawning reach) (Harding et al., 2019). Besides their well-documented effects during spawning seasons, inputs of necromass can have residual effects across seasons, with spawning salmon biomass in the autumn predictive of bird density and diversity in estuaries the following summer (Field and Reynolds, 2011). Findings such as these illustrate the important and complex roles necromass and phenology-based mortality play in aquatic ecosystems.

Although few aquatic insects have evolved to feed directly on carrion, the influx of nutrients from salmon carcasses, and resulting increases in primary production, can increase aquatic insect density by 8–25 times in artificial and natural streams where carcasses are present (Wipfli et al., 1998; Fenoglio et al., 2014). Isotopic studies have shown that salmon-derived carbon and nitrogen is incorporated into both primary producers and invertebrate feeding groups that consume microbes (e.g., filterers and grazers) (Bilby et al., 1996; Johnston et al., 1997; Guyette et al., 2014). While salmon remains the best studied example of phenology-based mortality (Schindler et al., 2003), they are by no means the only group of aquatic animals with programed mortality that leads to cascading effects in aquatic ecosystems. In addition to bony fish and invertebrates (see below), carcasses of other aquatic organisms, such as the sea lamprey (*Petromyzon marinus*), represent important nutrient sources, connect marine and freshwater systems, and can stimulate primary productivity (Weaver et al., 2018). While catadromous organisms which migrate to the ocean to spawn, [e.g., eels (*Anguilla* spp.)], also link marine and freshwater systems; how their behavior impacts marine ecosystems remains largely unknown. However, their spawning and subsequent death likely introduces considerable nutrients into otherwise oligotrophic environments where spawning occurs (e.g., Sargasso Sea).

STOCHASTIC AND EPISODIC MASS MORTALITY AND DECOMPOSITION

Vertebrate Carrion Mass Mortalities

One of the most striking examples of vertebrate mortality altering aquatic ecosystem comes in the form of episodic mass death, and the resulting carcasses that undergo decomposition, due to stochastic factors not easily predicted in nature (Fey et al., 2015). Mass fish kills are one of the most visible forms of this kind of mass mortality, where tens of thousands of fish may die within a short (e.g., hours to days) period of time, causing mass decomposition in the water and on banks of aquatic ecosystems (Ochumba, 1990; Thronson and Quigg, 2008). Fey et al. (2015) reported fish mass mortality events made up about 56% of all mass mortality events reported in scientific literature since the 1940s (*N* = 727). Episodic mass mortalities can be the result of stochastic changes in physical–chemical conditions (Cooper, 1993), toxic algal blooms (Hallegraeff, 1993), disease (Grizzle and Brunner, 2003), pollutants (Cooper, 1993), and other unknown factors. In many instances these conditions are the result of eutrophication over many years (Vollenweider, 1970; Harper, 1992; Nixon, 1995), or through punctuated high inputs of nutrients, like in the case of hippopotamus urine and feces (Subalusky et al., 2015) or mass drownings of wildebeest (Subalusky et al., 2017; Dutton et al., 2018). There are also other forms of vertebrate mass mortality that occur in aquatic ecosystems (Fey et al., 2015). For instance, annual mass drownings of wildebeest (Subalusky et al., 2017), aquatic reptiles (Rachowicz et al., 2006) and mammals that succumb to disease (Osterhaus et al., 1997; Kennedy, 1998) have both short- and long-term effects on ecosystem function. Fey et al. (2015) also provide an excellent quantitative assessment of mass mortality events worldwide, showing an increase in their occurrence for mammals, birds, amphibians, reptiles, fish, and marine invertebrates since 1940.

Invertebrate Carrion Mass Mortalities

Much like cicada emergences that have been quantified to have significant impacts on terrestrial food webs and ecosystems (Yang, 2004), mayflies (Ephemeroptera), midges (Gratton et al., 2008), salmonflies (Plecoptera) (Walters et al., 2018; Wesner et al., 2019), and other aquatic insects (Baxter et al., 2005) emerge in masses as adults to mate and die, with their carcasses falling back to the aquatic habitat or into the adjacent riparian zones and inland landscapes (Gergs et al., 2014). When these mass emergences cross habitat or ecosystem boundaries (e.g., from a stream or lake onto the shoreline), such cross-ecosystem resource subsidies (Polis et al., 1997) can represent significant nutrient and energy pulses (Polis, 1994; Polis and Hurd, 1996). For example, Wesner et al. (2019) reported that for several stream sites with massive emergences of salmonflies (*Pteronarcys californica*), the resulting insect carrion deposition on the adjacent shore over only a single week was equal to or greater than annual atmospheric nitrogen and phosphorus deposition and the annual secondary production of all terrestrial insects from that watershed. Such contributions to the detrital pools of adjacent ecosystems have not been well studied, especially compared to the living emerged insects that are consumed by predators. Indeed, more studies are needed to better quantify the contributions of necromass originating from aquatic ecosystems and acting as resource subsidies to the decomposition budgets of adjacent ecosystems.

Mass mortalities of aquatic invertebrates, beyond what was discussed above with parasite-mediated terrestrial insect drownings, also affect in-stream and riparian communities and ecosystem properties. While not as well documented as fish subsidies, invertebrate mass mortalities have significant and sometimes long lasting effects on aquatic ecosystems since many of the invertebrate species, like mussels, have important functional roles (Vaughn, 2018), but also can contribute mass subsidies of highly recalcitrant structures, such as shells of molluscs (McDowell et al., 2017; DuBose et al., 2019). Beyond the pulsed effects of nutrient release related to rapid soft tissue decomposition of bivalves, Wenger et al. (2019) estimated that mussel shells from mortality events may have once provided about 1% of total phosphorus load in streams and rivers of the southeastern United States.

McDowell and Sausa (2019) reviewed the effects of mass mortality effects of the invasive bivalves *Corbicula* sp., the zebra mussel *Dreissena polymorpha*, the golden mussel *Limnoperna fortunei*, and the Chinese pond mussel *Sinanodonta woodiana*. Low and high water temperatures and water levels were the leading causes of mass mortality of both invasive and native species. There were short-term (i.e., over days) nutrient pulse releases associated with *en masse* soft tissue decomposition and longer-term microhabitat effects in the form of remaining shells both as part of the benthic substrata but also on stream and river banks. For instance, a mass mortality event of about 100 million *C. fluminea* contributed an estimated 751 kg of carbon, 180 kg of nitrogen, and 45 kg of phosphorus to a stream over the course of several days (McDowell et al., 2017). The dead and dying bivalve carcasses may also serve as food resources for local scavengers like fish, invertebrates, and birds (Mouthon and Daufresne, 2006; McDowell and Sausa, 2019). Mass mortality of *C. fluminea* left on stream banks attract a wide diversity of terrestrial invertebrates (Novais et al., 2015) and below ground nutrients (Novais et al., 2017), suggesting that bivalves washed onto banks during floods can become pulsed resources subsidies for adjacent habitats.

Mass mortality events also occur throughout the world in marine and estuarine habitats, and not surprisingly changing climate patterns and warming temperatures are increasing their magnitude and frequency (Coma et al., 2009). While not always independent of increases in water temperature, diseases also contribute to marine invertebrate mass mortality (Harvell et al., 1999). Not matter what the cause, marine invertebrate mass mortality has significant effects on coral reef and intertidal ecosystems by affecting both hard and soft corals, benthic burrowers and filterers with cascading effects on upper trophic level consumers (Knowlton, 2004). These massive death events are often extensive. For instance, in 2016, one of the three pan-tropical mass coral bleaching events occurred in the Great Barrier Reef of Australia as a result of a significant marine heat wave, where 90% of the surveyed reefs suffered mortality (Hughes et al., 2017). The ecosystem consequences of this event included significant community restructuring, functional changes, and widespread declines in consumers resulting directly from the water temperature increases, but also in relation to coral loss (Stuart-Smith et al., 2018). Increased frequency and magnitudes of such global weather events will have widespread ecological impacts on all ecosystems, with mass mortalities contributing to many of the most negative effects of a changing climate.

SUMMARY

Death and decomposition occur in all ecosystems, but the extent and magnitude of the resulting necromass varies in space and time. Some forms of necromass come from within the system, as part of the life cycles and life histories of the resident organisms (e.g., planktonic snow in a lake or ocean or insects in a stream) or during mass deaths resulting from changing and intolerable habitat conditions (e.g., fish kills and coral reef bleaching). Other forms of necromass subsidies come from outside of the system, sometimes as migratory fish (e.g., salmon) or parasitized terrestrial insects that have been behaviorally hijacked to enter aquatic habitats where they drown. In all cases, the once living biomass becomes an often significant and functionally important component of the detrital pool. This component of heterotrophically derived necromass has historically been difficult to quantify due to the rapid turnover of such labile resources, and has arguably been overlooked or underappreciated in many ecosystem level energy and nutrient budgets. Recent work has provided conceptual models and methods for improving the ability to identify, quantify, and better study how carrion resource subsidies affect aquatic ecosystems, ranging from small, headwater streams to saltmarshes and mangroves, to the deep oceanic abyss and enormous stretches of coastline habitats around the world. Furthermore, while studies of megafauna carrion (e.g., whales and whale sharks) have demonstrated significant impacts to deep ocean habitats, much less is understood for other groups of animals. Quantifying the energy, nutrient, and foodweb effects of autochthonous and allochthonous carrion resulting from non-consumptive morality, phenology-based mortality and stochastic and episodic mortality events will allow broader assessments of all necromass contributions in aquatic ecosystems. With advances in geographical (e.g., drones and satellite imagery), genomic (e.g., next-generation sequencing), and other forms of technology (e.g., cell phone cameras and associated software) it will become increasingly feasible to quantify baseline levels of carrion to more fully evaluate how this resource subsidy affects ecosystem energy and nutrient dynamics.

AUTHOR CONTRIBUTIONS

MB, JR, and GL performed the literature search, and writing and editing of this review.

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Quantitative Food Webs Indicate Modest Increases in the Transfer of Allochthonous and Autochthonous C to Macroinvertebrates Following a Large Wood Addition to a Temperate Headwater Stream

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Headwaters suffer from reduced leaf and wood inputs and retention capacity from historical land actions like watershed logging and agriculture. When in-stream wood is reduced, stream retention capacity declines and subsequent changes in streamwater flow-paths and patterns of deposition alter decomposition and primary production that influence secondary invertebrate production via modified habitat and resources. Wood additions are commonly used as stream restoration tools for habitat improvements that can restore or strengthen food web connections; however, changes in carbon (C) flow through food webs are rarely measured because of time and expense. We quantified allochthonous and autochthonous C flow through aquatic macroinvertebrate communities 1 year before and 2 years after an experimental addition of large wood, compared to macroinvertebrates in an upstream control, in a temperate headwater stream. We predicted wood additions increase macroinvertebrate consumption and assimilation of allochthonous and autochthonous C through retention of leaves and altered flow-paths that expose more gravel and cobble for periphyton colonization. Macroinvertebrate allochthonous C assimilation tended to increase in years with greater organic matter retention and autochthonous C increased with more exposed gravel and cobble across seasons and between reaches. While the effect of wood addition on C flow through the macroinvertebrate community was minimal, it increased by ∼20% relative to the control from an increase in production and C assimilation of common mayfly and caddisfly scrapers, *Baetis* and *Glossosoma*. Because the amount of organic matter retained and coarse substrate exposed corresponded with C form and amount consumed, restoration of large wood has the potential to increase organic matter C trophic transfer.

Keywords: headwaters, large woody debris, restoration, stable isotopes, organic matter

INTRODUCTION

Freshwater ecosystems cover only a small fraction of Earth's surface but receive at least 1.9 Pg carbon yr^{-1} from terrestrial ecosystems. At least 50% of these carbon (C) inputs are stored in or emitted from freshwaters (Cole et al., 2007). Consequently, cross-ecosystem terrestrial-aquatic exchanges are essential for understanding global C cycling (Cole et al., 2007). Stream food webs are strongly influenced by leaf-litter and wood from adjacent riparian areas. Changes in riparian detrital quality and quantity dictate aquatic biological community structure, organismal growth, and organismal lifecycle completion rates, described as organismal performance (Wallace et al., 1997b). Collectively, this community performance governs aquatic ecosystem functions like secondary production and decomposition (Webster et al., 1997). In turn, in-stream secondary production and decomposition govern the capacity of small streams to transfer and transport C that subsidizes downstream and riparian communities.

Human actions in forested watersheds can change the timing or amount of allochthonous and autochthonous C that affect the organisms available to consume and transfer C (Cummins et al., 1989). For example, greater retention of allochthonous material in streams stimulate growth of fungi and bacteria, and organic matter consumption by macroinvertebrates (Richardson, 1991; Negishi and Richardson, 2003; Tiegs et al., 2008), increasing the relative amount of allochthonous versus autochthonous C transferred through the stream food web (Rosemond et al., 1993). However, increases in water velocity can increase exposure of large substrates subsequently covered in sand and stimulate periphyton growth (Kail, 2002), increasing the relative contribution of autochthonous C to secondary consumers like macroinvertebrates (McNeely et al., 2007). Large wood results in both organic matter retention and substrate sorting via modified flow paths. Therefore, adding wood to streams can change available habitat and food resources for aquatic biota. The concurrent increase in autochthonous and allochthonous C in low-production, forested streams could lead to an overall increase in C contributions to secondary macroinvertebrate production, resulting in greater trophic transfer and more trophic linkages.

Attempts to restore headwater streams through a bottom-up organic matter addition (e.g., addition of large wood) provide stability and greater resource availability that could also alter the relative terrestrial- and instream-derived C eaten and assimilated by aquatic heterotrophs (e.g., Rosi-Marshall and Wallace, 2002). Forested headwater streams have historically been considered to be strongly influenced by the volume and timing of terrestrially derived allochthonous material delivered to the stream (Polis and Strong, 1996; Wallace et al., 1997b; Moore et al., 2004). However, algae can also be seasonally important in many temperate streams (Finlay, 2001; Hall et al., 2001). The alternating increase of algae in winter when the canopy is more open and leaf litter inputs retained in autumn may act to stabilize the food web when resources may otherwise be scarce (Power et al., 1988; Polis and Strong, 1996; Moore et al., 2004; Guo et al., 2016). We are unaware of any studies that have quantified allochthonous C (i.e., terrestrially derived) versus autochthonous C (algal-derived) following in-stream wood addition.

Ecosystem processes that restorations aim to affect include rates of nutrient uptake (Sudduth et al., 2011), organic matter retention, and decomposition (Lepori et al., 2005; Frainer et al., 2017). These functional metrics are especially useful in restoration studies because they reveal how changes in physical structure may influence the rate, and pathway of energy or elements flowing through an ecosystem. Stable isotopes can be used to identify the source and amount of C assimilated by a target community and are increasingly used in restorations (for example, Fry, 2002; Kennedy et al., 2005; Lepori et al., 2006). For example, Lepori et al. (2006) added boulders to several streams in Sweden that increased detrital retention, but did not result in consumers $\delta^{13}C$ more similar to the retained detritus.

Still, stable isotopes may identify food source assimilated and can be used in combination with measures of community structure and secondary production to develop quantitative food webs (e.g., Rosi-Marshall and Wallace, 2002). Quantitative food webs can reveal consumer-level controls on ecological processes and illustrate changes in trophic structure (Rosi-Marshall and Wallace, 2002), community assemblage, and nutrient flow (Cross et al., 2007)following ecosystem restoration. Tracking pathways of energy flow through the food web integrates changes in food resource assimilation, community structure, survival, and production (Benke and Wallace, 2011). Carbon flow measurements are commonly used to test ecological theory (e.g., Cross et al., 2007), but have not been used to assess restorations.

We quantified allochthonous and autochthonous C assimilation in macroinvertebrates before and after experimental wood addition in a Michigan headwater stream using natural abundance of C isotopes for macroinvertebrates and their food resources. Then we combined estimates of C assimilation with secondary production (Entrekin et al., 2009) to calculate trophic basis of production (Benke and Wallace, 1997). Finally, by using assumed assimilation efficiencies, we were able to back-calculate the amount of allochthonous and autochthonous C consumed (expressed as a rate) and compare that C flow with the amount available. Our previous work in this stream showed that total macroinvertebrate secondary production was low before wood addition, but increased by ∼25% 2 years after wood addition, resulting in a statistically significant increase in invertebrate biomass and greater secondary invertebrate production (Entrekin et al., 2009). Here, we sought to quantify how assimilation, trophic basis of production and C allochthonous and autochthonous C flowing through the macroinvertebrate food web changed after wood was added. We predicted wood addition would increase consumption and assimilation of allochthonous and autochthonous C by macroinvertebrates through an increased retention of leaf litter, and the exposure of sand-covered large inorganic substrates for periphyton colonization.

STUDY SITE

State Creek is a 1st-order stream draining 3.9 km^2 in the Ottawa National Forest in the Ontonagon River basin in the Upper Peninsula of Michigan, United States (46° 28′N, 89° 1′W). We began sampling monthly in May 2003, 1 year before wood addition (Y0) and continued for 1 (Y1) and 2 (Y2) years after wood addition in both the wood-added (treatment) and the upstream control reach. Treatment and control reaches were separated by a 50 m distance to promote some independence between sites, while reducing differences associated with longitudinal changes. We measured no difference in the amount of in-stream wood between the control and treatment reaches prior to the wood addition (Entrekin et al., 2007). The stream section we studied had 90% canopy cover during spring and summer and 75% canopy cover in autumn and winter, an average bank-full width of 2.4 m in the control reach and 2.5 meters in the treatment reach, an average water depth at base flow of 13 cm in the control reach and 12 cm in the treatment reach, and an average discharge in the control reach of 64 and 67 L s^{-1} in the treatment reach (for more details see Entrekin et al., 2007; Hoellein et al., 2009). The stream flows through a managed, second-growth forest with intact, but young, riparian vegetation in a catchment of 95% deciduous forest that was last logged in 1967. Riparian trees include *Populus tremuloides* Michx (trembling aspen), *Acer rubrum* L. (red maple), *Acer saccharum* Marsh. (sugar maple), *Betula papyrifera* Marsh. (paper birch), *Tsuga canadensis* L. (hemlock), *Pinus alba* L. (white pine), with a thick understory of *Alnus serrulata* Alt. (tag alder). The study stream had low in-stream large wood density (13 pieces/100 m stream length) and low storage of coarse benthic organic matter [annual average of 146 ± 70 (SE) g AFDM m^{-2}] before wood addition (Cordova et al., 2007; Entrekin et al., 2007) from a history of region-wide logging and shale mining (Webster et al., 2008).

In May 2004, we added 25 logs (each 2.5 m long × 0.5-m diameter) of big tooth aspen *P. grandidentata* Michx., purchased from a nearby tree farm, haphazardly to a 100-m stream reach, while maintaining a 100 m upstream control reach. After 2 years in the stream, 15 of the 25 logs moved. Most of the added logs moved less than two meters with one moving the farthest at 18 m (G. Lamberti unpublished data). None of the added wood moved out of the study reaches. We did measure an increase in the amount of organic matter retained and more exposed cobble from a localized increase in water velocity caused by the added logs (Entrekin et al., 2008).

MATERIALS AND METHODS

Habitat Characteristics

Standing crops of coarse (CBOM) and fine (FBOM) benthic organic matter were measured from five 804-cm^2 benthic cores sampled in each reach on each sampling date; CBOM was separated from FBOM using a 1-mm sieve. After CBOM was removed from the corer, a FBOM slurry was made by stirring the sediment in the core and then subsampled using a 160 mL specimen container. Subsamples were stored on ice until processing. In the laboratory, samples for CBOM were dried at 60°C, sorted by organic matter type (leaves, moss, and wood), and weighed. A subsample of each organic matter type was then combusted at 550°C, and reweighed to determine ash-free dry mass (AFDM; Benfield 2006). For FBOM, subsamples were filtered onto glass fiber filters (GF/F), dried at 60°C, weighed, combusted at 550°C, and reweighed for AFDM.

On each sampling date, we also measured discharge and velocity from the dilution of a conservative tracer during concurrent measurements of whole-stream nutrient uptake rates (Hoellein et al., 2007). Water temperature was recorded at the bottom of each stream reach hourly from May 2003–May 2006 using HOBO® data loggers (Onset Computer Corporation, Bourne, MA, United States). We also surveyed benthic habitat using transects spaced every 5 m (perpendicular to flow) in both reaches in May and August of 2003 (before wood addition) and in May, July, and November 2004–2006 (after wood addition). Sediments were categorized using the Wentworth scale (Minshall and Rugenski, 2006). Inorganic sediments were classified as boulders, gravel and cobble, and sand, while organic substrates were moss, CBOM (>1 mm), silt, small wood (<10 cm), and large wood (>10 cm). Measurements were recorded every 20 cm across the channel. We calculated percent cover for each substrate at each transect-scale, and as the mean of each category across all transects combined (i.e., reach-scale).

Measuring $\delta^{13}C$ Natural Abundance Signature

We measured $\delta^{13}C$ values for the most productive taxa that collectively represented ∼90% of the total macroinvertebrate community production (Entrekin et al., 2009). Macroinvertebrate abundance, community composition, and secondary production were measured from monthly (none collected in January) Hess (32 cm diameter, 250 μm-mesh) samples (5 per stream reach) in the treatment and control reaches for the 5-year study period. For secondary production, size-frequency histograms were developed for each taxon and corrected using cohort production intervals. For rare taxa, we used either production to biomass ratios we developed or published values (Entrekin et al., 2009). We then used a sub-set of those individuals for stable isotope analysis. Samples were preserved in 6–8% formalin, which we note may result in a 1–1.65‰ systematic depletion of $\delta^{13}C$ across taxa (Sarakinos et al., 2002; Bicknell et al., 2011). However, a comparison of $\delta^{13}C$ differences between frozen and formalin-preserved samples for four dominant taxa in one season showed no consistent change (S. Entrekin, unpublished data). Despite some inorganic C, we did not acidify samples because inorganic C was low and acidifying can lead to fractionation (Schlacher and Connolly, 2014). To measure C stable isotopes of macroinvertebrates, we selected late instar taxa from March (late winter), May (spring), June or July (summer), and November (autumn) for the three study years to represent possible seasonal changes in taxa. Thus, our macroinvertebrate sampling incorporated seasonal variation in macroinvertebrate diet as well as any potential changes due to the wood addition.

For each stable isotope measurement, the number of individuals representing a single taxon varied based on an individual's mass (i.e., 2–20 individuals). When possible, we used individuals from at least three different replicate Hess cores that were taken haphazardly along each 100-m reach to incorporate reach-scale variation and expressed as averages with standard error (see **Supplementary Appendix 1**). Finally, we measured the $\delta^{13}C$ signature of macroinvertebrate food resources including conditioned leaves (i.e., leaf litter colonized by bacteria and fungi) and algae from grab samples in the selected months. Conditioned leaves were collected along each stream reach from State Creek in spring, summer, autumn, and winter and frozen until analysis. Periphyton samples were also collected seasonally by scraping multiple rocks throughout the stream reach.

Macroinvertebrate taxa and food resources were dried at 60°C, ground to a fine powder, and analyzed on a Finnigan Delta Plus Stable Isotope Mass Spectrometer. Stable isotope values were expressed in δ notation as the difference in parts per thousand (‰) from a standard (PeeDee Belemnite), using the calculation: $\delta^{13}C = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000$; where R is $^{13}C/^{12}C$. Our attempts at silica separation (Hamilton et al., 2005) were unsuccessful in isolating low amounts of algae (mostly diatoms) from periphyton biofilm scrapings. Therefore, we used the $\delta^{13}C$ value of a known grazer, the caddisfly *Glossosoma* sp., as the presumed proxy for periphyton, as has been done in other studies (e.g., Finlay, 2001). *Glossosoma* gut contents were examined each season to verify the presence of algae; however, small amounts of allochthonous C may have been assimilated that would result in an underestimate of the contribution of autochthonous C (to the invertebrate community diet).

Our first goal was to partition allochthonous (i.e., leaf litter) from autochthonous (i.e., in-stream algae) C assimilation by macroinvertebrates as indicated by $\delta^{13}C$ signatures using a two-source mixing model rather than using gut content analysis. Therefore, no other food resources were used in the mixing model because leaf litter and epilithic algae (or periphyton) represented the end members (allochthonous versus autochthonous food resources) in this food web. We used the model IsoSource and IsoError from the Environmental Protection Agency[1], which has the advantage of accounting for replicate sample variation (Phillips and Gregg, 2001). The $\delta^{13}C$ average of conditioned leaf litter was −29.98 ± 0.01‰, and $\delta^{13}C$ of algal signature of *Glossosoma* sp. was 36.94 ± 0.2‰ (upper confidence interval = 1 and lower confidence interval = 0.97) across our sample period, indicating separation of the two C sources. $\delta^{13}C$ signatures were not corrected for C fractionation rates as they are likely low and unpredictable (McCutchan et al., 2003).

Relative Allochthonous and Autochthonous C Assimilation and Their Contribution to Production

Then, we used a modified method to quantify the trophic basis of production described by Benke and Wallace (1997, 2011) where the proportional contribution of allochthonous and autochthonous C to assimilation for each taxon, as indicated by stable isotopes, was multiplied by the total production of that taxon to determine the contribution to secondary invertebrate production or the trophic basis of production. The contribution of allochthonous and autochthonous C to production was then calculated for each dominant macroinvertebrate taxa and summarized for each functional feeding group (FFG). The advantage of this method was that assumptions about assimilation efficiency were not needed because natural abundance of isotopes reflect assimilation. However, we were not able to estimate total amount of resources consumed. Therefore, to quantify how much autochthonous and allochthonous food was consumed (i.e., in units of AFDM per m^{-2} $time^{-1}$), we divided secondary production by published assimilation efficiencies for detritus and algae and then multiplied that by published net production efficiency (Benke and Wallace, 1997; Hall and Meyer, 1998; Rosi-Marshall and Wallace, 2002) to get autochthonous and allochthonous C consumption (Benke and Wallace, 1997):

Algal or detrital C consumption = (secondary production/assimilation efficiency) *net production efficiency

where secondary production is expressed as mg dry mass per m^{-2} yr^{-1}, assimilation efficiency is a proportion (detritus = 0.1 or algae = 0.3), and net production efficiency is a proportion (NPE = 0.5).

Statistical Analyses

We used a Before-After-Control-Impact Analysis of Variance (BACI-ANOVA) (Stewart-Oaten et al., 1986; Underwood, 1992) with a Tukey *post hoc* when significance at $\alpha = 0.05$ was met to test for changes in $\delta^{13}C$ and the contribution of allochthonous versus autochthonous C for the macroinvertebrate community, functional feeding groups, and for individual taxa. Pearson product moment correlations were used to explore the relationship among $\delta^{13}C$ values and environmental parameters that may contribute to variation across stream reaches through time (e.g., organic matter standing crops and % substrate cover). All data were tested for conformance to homogeneity of variance using Levene's test and assumptions of ANOVA using Kolmogorov-Smirnov tests, and transformed when assumptions of normality were not met. All analyses were performed with SAS software (v. 8.02, SAS Institute Inc., Cary, NC, United States).

The amount of C consumed, assimilated and supporting secondary production (i.e., flux or flow) were not replicated measurements; therefore, we did not conduct statistics, but we present and describe the effect size as the difference between the treatment and control reaches before and after wood addition.

RESULTS

Changes in $\delta^{13}C$ Signatures After Wood Addition

$\delta^{13}C$ ranged from ∼−25‰ to −37‰ across all macroinvertebrate taxa (**Supplementary Appendix 1**). We were surprised that overall average macroinvertebrate $\delta^{13}C$ remained mostly unchanged following wood addition (**Figure 1**). Before wood

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[1] http://www.epa.gov/wed/pages/models/stableIsotopes/isotopes/isoerror1_04.htm

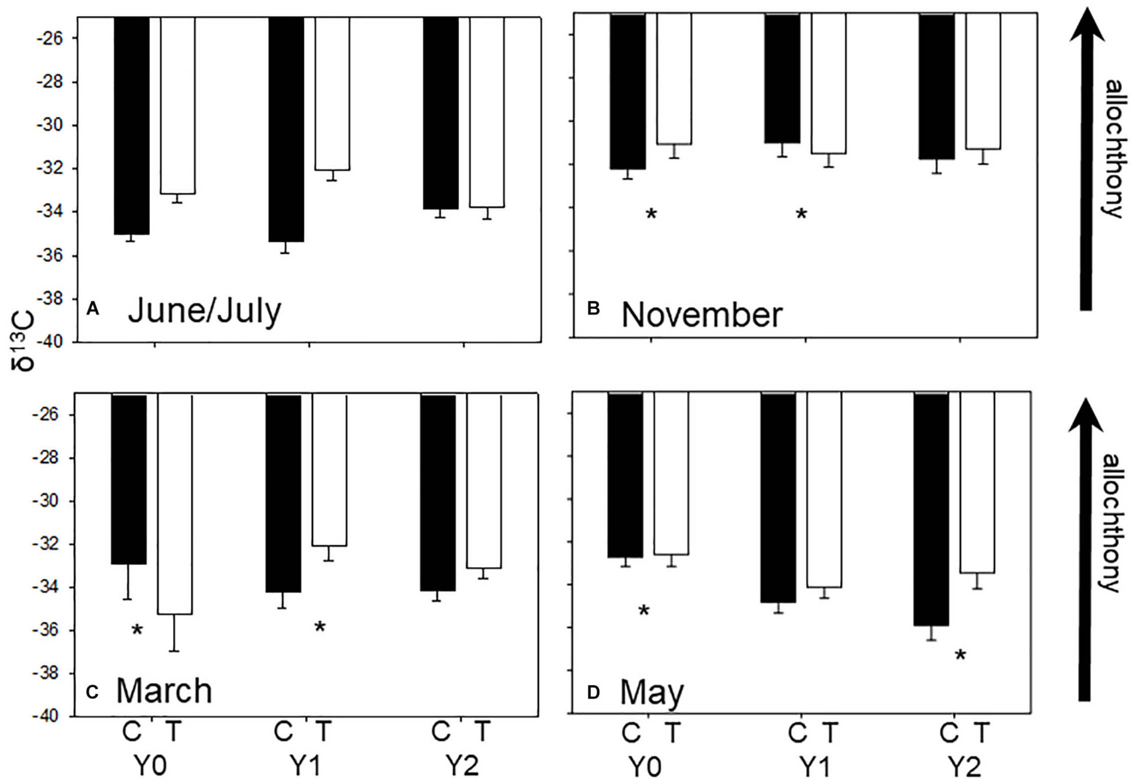

FIGURE 1 | Average (±SE) seasonal δ^{13}C values in each stream reach 1 year before (Y0) and 1 (Y1) and 2 years (Y2) after wood addition in control (C, light bars) and treatment (T, dark bars) in State Creek (Michigan). Dashed lines are average leaf litter δ^{13}C values. * Indicates a significant interaction between control and treatment reaches using ANOVA. **(A)** Are average seasonal δ^{13}C values from June/July, **(B)** are average seasonal δ^{13}C values from November, **(C)** are average seasonal δ^{13}C values from March and **(D)** are average seasonal δ^{13}C values from May.

addition, the mean (±1 standard error) δ^{13}C signature across all macroinvertebrate taxa differed by ~1‰ between the control and treatment reaches (**Figure 1** and **Table 1**). The pattern remained after wood addition, and there was no difference in community-level δ^{13}C between the treatment and control reach in Y1 ($F_{3,12} = 0.42$, $P = 0.74$) or Y2 ($F_{3,12} = 0.23$, $P = 0.87$) (**Table 1**). Similarly, there was no difference in functional feeding group and taxa-specific δ^{13}C between reaches after wood addition in Y1 or Y2 (**Table 1**).

Seasonal Changes in δ^{13}C Signatures

Macroinvertebrate δ^{13}C signatures reflected seasonal variation in resources as expected in a temperate headwater stream with signatures closer to leaf-litter in autumn (**Supplementary Appendix 1** and **Figure 1B**) and farther from leaf-litter in March (**Figure 1C**) when canopy is more open. Community mean δ^{13}C values were different among seasons ($F_{3,8} = 13.54$, $P < 0.001$) with greater δ^{13}C value of −31.79‰ (i.e., closer to leaf litter signature) in autumn (November) and a lower ~−33.5‰ δ^{13}C in winter, spring, and summer (i.e., farther from leaf litter signature) (**Figure 1**, Tukey's test $P < 0.001$).

The δ^{13}C signatures of many individual taxa responded to wood addition differently depending upon taxonomic identity and season, although not necessarily in a predictable manner (**Supplementary Appendix 1** and **Table 1**). For example, *Tipula*, an obligate shredder, tended to have δ^{13}C closer to leaf litter in the treatment reach relative to the control in Y2 compared to their difference in Y0. A dominant filterer, *Simulium*, had lower δ^{13}C in the treatment reach in May of Y2 compared to the difference between the control and treatment reach in Y0 (**Supplementary Appendix 1**, $F_{3,4} = 77.77$, $p < 0.001$). In addition, the predatory stonefly, *Isogenoides*, had greater δ^{13}C in the treatment reach in autumn of Y1 compared to the difference between the control and treatment reach in Y0 (**Table 1**, $F_{3,5} = 11.7$, $p = 0.01$). *Baetis*, among the most productive facultative scraper and gatherers, had greater δ^{13}C in the treatment reach in Y2 winter compared to the difference between the control and treatment reach in Y0 (**Supplementary Appendix 1**, $F_{3,7} = 18.6$, $p = 0.001$).

Variation in Macroinvertebrate Functional Feeding Group C Assimilation Related to Substrate Composition and Organic Matter Standing Stocks

We correlated allochthonous C assimilation of average community and functional groups with substrate type to identify environmental factors influencing variation across stream reaches and season (**Figure 2**). The overall % allochthonous C assimilation at the community level was not explained

TABLE 1 | Mean annual δ^{13}C values for each taxon.

	Y0		D	Y1		D	Y2		D
	C	*T*	*T–C*	*C*	*T*	*T–C*	*C*	*T*	*T–C*
Shredders									
Amphinemura sp.	−34.70	−33.90	0.80	−36.70	−34.50	2.20	−34.90	−34.40	0.50
Capniidae	−31.10	−31.30	−0.20	−28.60	−30.30	−1.70	−33.20	−31.80	1.40
Nemoura sp.	−37.80	−33.20	4.60	−38.20	−34.10	4.10	−34.10	−34.60	−0.50
**Tipula* sp.	−29.00	−26.70	2.30	−28.20	−28.90	−0.70	−29.30	−29.10	0.20
Mean	−33.15	−31.28	1.88	−32.93	−31.95	0.98	−32.88	−32.48	0.40
Gatherers									
Diamesinae	−36.80	−34.30	2.50	−34.80		34.80	−35.30	−33.60	1.70
Ephemerella spp.	−33.00	−31.60	1.40	−34.80	−31.10	3.70	−35.40	−31.90	3.50
Eukiefferiella spp.	−36.40	−34.00	2.40	−38.40	−36.30	2.10	−36.20	−35.80	0.40
Hesperoconopa sp.	−31.30	−30.40	0.90	−32.40	−30.80	1.60	−32.50	−31.80	0.70
Parametriocnemus sp.	−30.20	−30.10	0.10	−31.40	−29.10	2.30	−29.00	−29.50	−0.50
Tanytarsus spp.	−32.50	−30.10	2.40	−31.60	−29.60	2.00	−34.40	−33.30	1.10
Oligochaeta	−29.80	−30.90	−1.10	−30.10	−29.90	0.20	−28.40	−29.10	−0.70
Mean	−32.86	−31.63	1.23	−33.36	−31.13	2.22	−33.03	−32.14	0.89
Scrapers									
**Baetis* spp.	−36.00	−35.50	0.50	−37.80	−34.90	2.90	−35.90	−34.70	1.20
Gossosoma sp.	−36.70	−36.00	0.70	−37.60	−36.70	0.90	−37.70	−37.40	0.30
Neophylax sp.	−38.20	−35.70	2.50	−38.40	−35.60	2.80	−38.40	−38.00	0.40
Rhithrogena sp.	−30.40	−31.30	−0.90		−35.10			−34.30	
Mean	−35.33	−34.63	0.70	−37.93	−35.58	2.36	−37.33	−36.10	1.23
Filterers									
Dolophilodes sp.	−31.90	−30.40	1.50	−29.40	−30.60	−1.20	−32.10		
Parapsyche sp.	−31.40	−29.80	1.60	−31.90	−30.80	1.10	−33.20	−30.70	2.50
Prosimulium sp.	−32.50	−33.50	−1.00	−32.60	−32.00	0.60	−31.70	−33.30	−1.60
**Simulium* spp.	−33.70	−32.20	1.50	−31.00	−31.60	−0.60	−30.90	−30.50	0.40
Mean	−32.38	−31.48	0.90	−31.23	−31.25	−0.02	−31.98	−31.50	0.48
Predator									
Ceratopogonidae	−32.60	−33.10	−0.50	−31.40	−30.90	0.50	−31.30	−31.90	−0.60
Dicranota sp.	−31.30	−32.20	−0.90	−32.00	−31.00	1.00	−30.70	−31.70	−1.00
**Hexatoma* spp.	−30.80	−29.70	1.10	−31.50	−29.70	1.80	−32.80	−28.10	4.70
Isogenoides sp.	−32.90	−31.90	1.00	−32.60	−31.90	0.70	−33.90	−33.70	0.20
Rhyacophila sp.	−32.70	−31.60	1.10	−31.00	−32.60	−1.60	−33.40	−31.20	2.20
Mean	−32.06	−31.70	0.36	−31.70	−31.22	0.48	−32.42	−31.32	1.10
Community mean	−33.15	−32.14	1.01	−33.43	−32.23	1.20	−33.53	−32.71	0.82

**Indicates a significant change in mean macroinvertebrate δ^{13}C value after wood addition. D is the difference in treatment (T) and control (C). B is before (Y0) and A is after (Y1 and Y2) wood addition. Functional groups are in bold.*

by any one variable, although variation between stream reaches was related to the proportion of sand making up the stream bottom (**Figure 2A**). The relative amount of allochthonous C assimilated by macroinvertebrate FFGs across season and reaches was mostly correlated to differences in available coarse substrate and organic matter standing crop. The overall proportion of allochthonous C assimilation by shredders, filterers, and gatherers was positively related to the mean annual coarse organic matter standing crop (**Figures 2B,C,E**). In contrast, scrapers, that feed mostly on inorganic substrates, ranged from less than 1 to 15% allochthonous C assimilation. The proportion of terrestrially derived C assimilated by predators closely tracked gatherer assimilation of allochthonous C (**Figure 2F**).

All Functional Feeding Groups Consumed Allochthonous and Autochthonous C Sources, but FFGs Consumed Different Amounts of the C Sources

Allochthonous and autochthonous-based C contributed roughly equal parts to macroinvertebrate production in the control and treatment reaches, but with little change following wood addition. Annual contribution of allochthonous- C ranged from 35 to 44% in the control reach and 41–56% in the treatment reach over the 3-year study (**Figure 3A**). The origin of C assimilated by FFGs differed among groups as expected. Autochthonous-based C contributed the most to scraper production, ranging from 70 to 100%. However, allochthonous-based C contribution decreased in scrapers in Y1 relative to the difference between the control and treatment in Y0 (**Figure 3B**). For shredders,

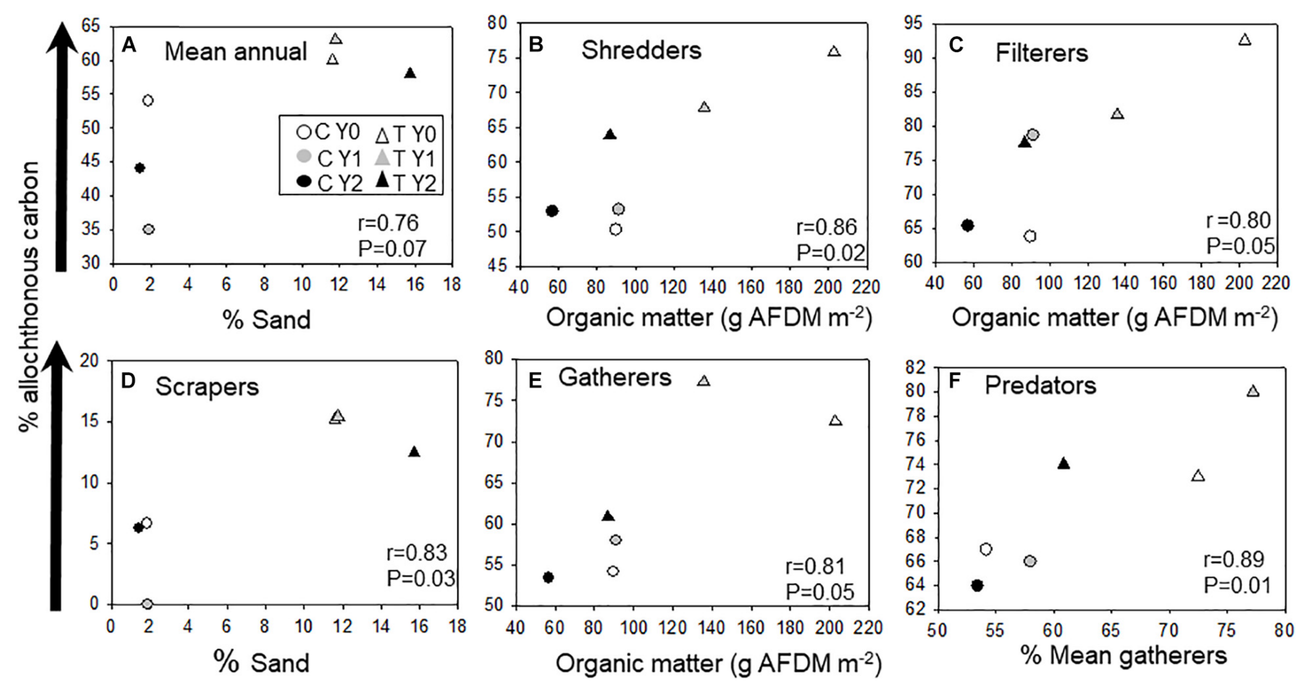

FIGURE 2 | Pearson product moment correlations between **(A)** annual average macroinvertebrate community δ^{13}C values, **(B)** macroinvertebrate shredders, **(C)** filterers, **(D)** scrapers, **(E)** filterers, and **(F)** predators and environmental variables. Control (C) upstream reach without wood addition and treatment (T) with wood added. Arrow indicates a gradient of the proportional increase in allochthonous C assimilation. Organic matter is coarse benthic organic matter fractions > 1 mm.

contribution of allochthonous C ranged from 50 to 90%, varying among years, reach, and taxa (**Figure 3C** and **Supplementary Appendix 1**). Contribution of allochthonous C to gatherer production increased in Y1 in the treatment reach relative to the difference between the control and treatment in Y0, ranging from 58 to 66% in the control reach and 72 to 88% in the treatment reach (**Figure 3D**). Allochthonous C contribution to filterer production increased in both reaches in Y1 and declined back to pre-wood addition values in Y2 (60–90%; **Figure 3E**). Allochthonous C contribution to predator production ranged from 62 to 77% and changed very little following the addition of wood (**Figure 3F**).

Actual Allochthonous-Based and Autochthonous-Based C Contributing to Macroinvertebrate Production

In the control reach, allochthonous and autochthonous production declined each year of the study. In contrast, allochthonous and autochthonous -based production in the treatment reach was identical in Y0 and Y2 but lower in Y1 (**Table 2** and **Figure 4A**). All FFG secondary production declined in Y1, while scraper and shredder production increased beyond Y0 values. Autochthonous-based scraper production was lowest in the treatment reach in Y1 and greatest in the treatment reach in Y2 (**Figure 4B**). Changes in scraper production in Y2 of the treatment reach were driven by an increase in mayfly *Baetis* production and C assimilation changed from mostly autochthonous to more allochthonous (**Table 2**). Allochthonous based shredder production was also lowest in Y1 and greatest in the treatment reach in Y2 from an increase in *Tipula* production (**Figure 4C**). Other FFGs did not show evidence of a treatment effect. Gatherer production was consistent across years in the control reach, but declined in the treatment reach, while filterer production declined across years in both reaches (**Figure 4E**). Finally, overall predator production and the ratio of allochthonous and autochthonous-based C contributions to production changed little across dates and reaches (**Figure 4F**).

Consumption of Allochthonous and Autochthonous- C

We calculated the amount of allochthonous and autochthonous C consumed by macroinvertebrates from published assimilation efficiencies to document changes in the amount of each resource flowing through the macroinvertebrate food web. Because leaf litter has a lower assimilation efficiency (0.1) than algae (0.3), macroinvertebrates must consume more allochthonous -based C to support their energetic demands (Benke and Wallace, 1997). Therefore, autochthonous-based C contributed a larger proportion to production, even though autochthonous consumption was less than allochthonous (**Figure 5A**). All functional feeding groups consumed both C sources. However, scrapers consumed relatively more autochthonous (**Figure 5B**) and filterers (**Figure 5C**) consumed more allochthonous C. Shredders (**Figure 5D**) and gatherers (**Figure 5E**) consumed mostly allochthonous C and contributed more to overall energy

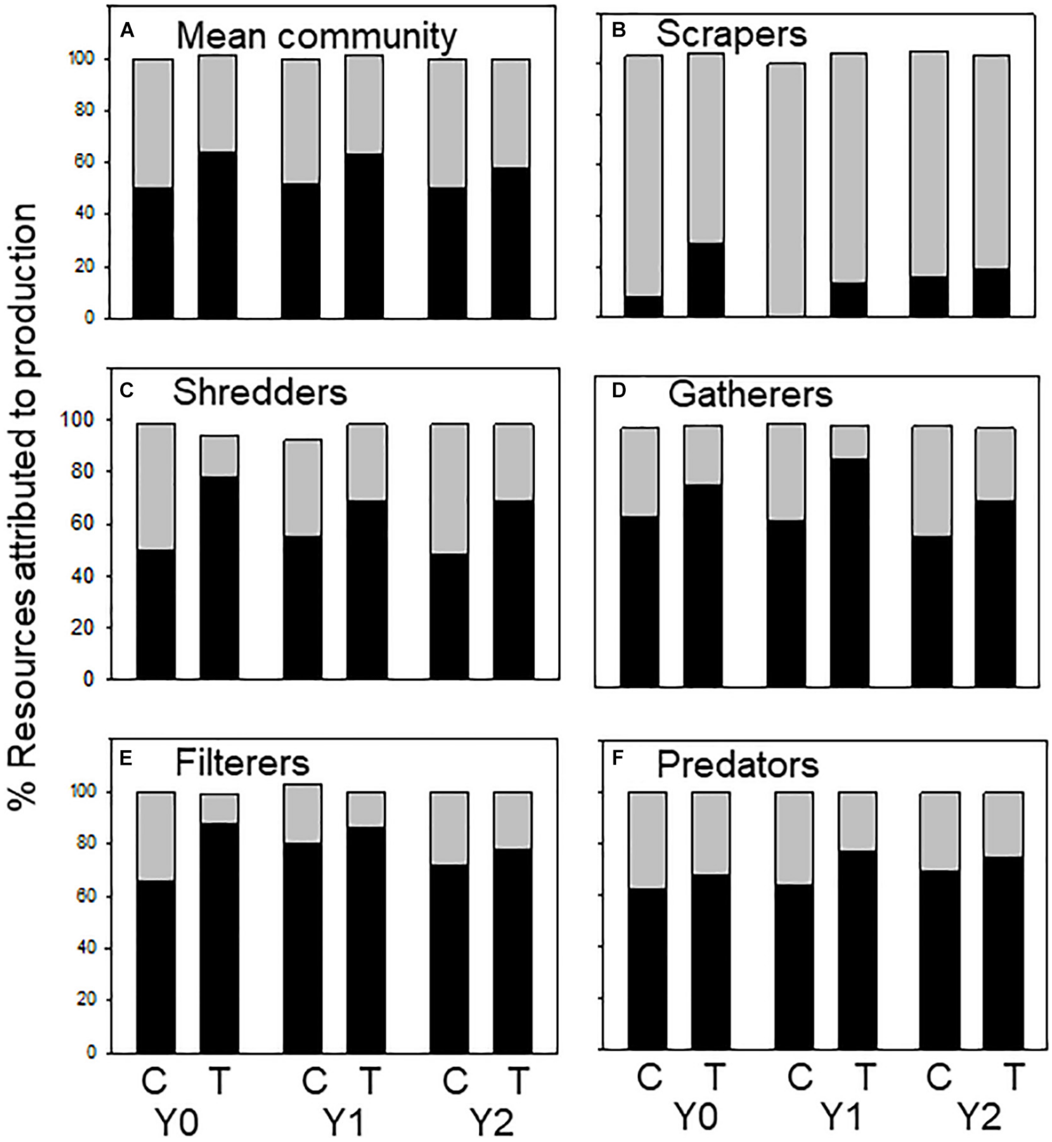

FIGURE 3 | Percent allochthonous (dark bars) and autochthonous (light bars) C contributing to macroinvertebrate secondary production (categorized by functional feeding groups) in an upstream control C and downstream wood-added reach (treatment, T) 1 year before (Y0) and 1 (Y1) and 2 years (Y2) after wood addition. Values may not equal 100% because percentages were averaged across taxa within functional feeding groups (FFGs). **(A)** % resources attributed to total macroinvertebrate production, **(B)** % resources attributed to scraper production, **(C)** % resources attributed to shredder production, **(D)** % resources attributed to gatherer production, **(E)** % resources attributed to filterer production, and **(F)** % resources attributed to predator production.

flow by consuming more material to support relatively greater secondary production (**Figure 5**).

Energy Flows From Autochthonous and Allochthonous-Based C

In the control reach, the dominant energy flows remained fairly consistent among Y0, Y1, and Y2 (**Table 3** and **Figure 6**). autochthonous C consumed by scrapers remained fairly evenly distributed between *Baetis* and *Glossosoma* in each year, with a slight increase to *Baetis* in Y2 (**Figure 6**). Allochthonous production also remained constant flowing mostly through the filtering black flies, *Simulium*, and to a lesser extent a net-spinning caddisfly, *Parapsyche*, with no changes from Y0 to Y2. In contrast, energy flow in the treatment reach showed much greater variation across years (**Figure 6**). Scraper basal C flow in Y0 was split between *Baetis* and *Neophylax* in the treatment reach and was predominantly autochthonous C sources. In Y1 and Y2 in the treatment reach, allochthonous C contributed the most to *Baetis* production in Y2 (**Figure 6**). For shredders, C flow through allochthonous C flow through *Tipula* was greater in Y2 than in Y0 or Y1.

TABLE 2 | Total allochthonous (alloch) or autochthonous (auto) carbon based production (mg AFDM m^{-2} y^{-1}) of each taxon calculated as production multiplied by the proportion of alloch or auto carbon source assimilated (estimated from $\delta^{13}C$ values).

	Y0						**Y1**						**Y2**					
	C		***T***		***D***		***C***		***T***		***D***		***C***		***T***		***D***	
Taxon	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**
Shredder																		
Amphinemura sp.	4.7	13.4	2.8	3.7	−1.9	−9.7	0.8	5.2	2.5	4.6	1.8	−0.6	3.5	9.2	2.0	2.4	−1.5	−6.9
Capniidae	11.7	3.8	3.7	0.9	−8.0	−2.9	32.7	0.0	24.5	1.6	−8.2	1.6	18.7	16.2	35.6	11.6	16.9	−4.7
Nemoura sp.	0.0	5.2	53.6	14.6	53.6	9.4	0.0	8.4	7.1	10.0	7.1	1.6	5.0	11.3	2.3	4.4	−2.8	−6.9
Tipula sp.	5.8	0.0	17.0	0.0	11.2	0.0	49.3	0.0	5.7	0.0	−43.6	0.0	27.9	0.0	88.2	0.0	60.3	0.0
Total	22.2	22.4	77.1	19.2	54.9	−3.2	82.8	13.6	39.8	16.1	−43.0	2.5	55.2	36.8	128.1	18.4	72.9	−18.5
Gatherer																		
Diamesinae	3.0	48.4	11.3	16.2	8.3	−32.2	12.8	12.5	0.0	0.0	−12.8	−12.5	3.9	12.5	7.1	6.6	3.2	−6.0
Ephemerella sp.	5.9	5.9	12.2	3.5	6.2	−2.3	24.8	53.1	57.4	23.3	32.5	−29.8	1.7	4.7	7.3	3.3	5.7	−1.4
Hesperoconopa sp.	23.1	5.5	76.9	5.3	53.8	−0.2	8.9	4.9	83.5	12.0	74.6	7.2	25.5	15.0	20.1	11.8	−5.4	−3.2
Oligochaeta	12.7	0.0	3.4	0.4	−9.3	0.4	20.6	2.7	8.5	0.2	−12.0	−2.4	15.6	0.8	17.2	4.6	1.6	3.8
Parametriocnemus sp.	0.4	0.0	0.3	0.0	−0.2	0.0	0.5	0.1	0.3	0.0	−0.2	−0.1	0.4	0.0	0.3	0.0	−0.1	0.0
Tanytarsus sp.	12.2	7.0	23.7	0.3	11.5	−6.7	16.2	8.5	6.9	0.0	−9.3	−8.5	16.4	30.3	7.0	6.4	−9.4	−23.9
Total	57.4	66.8	127.8	25.7	70.4	−41.1	83.8	81.7	156.6	35.5	72.8	−46.2	63.4	63.3	44.6	22.9	−18.8	−40.5
Filterer																		
Dolophilodes sp.	51.1	22.3	23.8	1.7	−27.4	−20.6	0.0	0.0	0.0	0.0	0.0	0.0	4.0	1.9	31.8	3.1	27.8	1.2
Parapsyche sp.	104.1	30.9	332.6	29.7	228.6	−1.1	80.0	26.8	202.3	16.0	122.3	−10.8	58.8	51.9	28.0	3.5	−30.9	−48.3
Prosimulium sp.	461.1	294.0	0.0	98.7	−461.1	−195.3	29.4	15.3	32.1	13.3	2.7	−2.0	5.9	2.0	2.3	2.1	−3.6	0.1
Simulium sp.	224.4	245.2	0.0	0.0	−224.4	−245.2	173.9	0.0	0.0	0.0	−173.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	840.7	592.4	356.4	130.2	−484.3	−462.2	283.3	42.1	234.4	29.3	−48.9	−12.8	68.8	55.7	62.1	8.7	−6.7	−47.0
Scraper																		
Baetis spp.	109.2	698.2	178.7	622.0	69.5	−76.2	0.0	414.8	89.1	292.6	89.1	−122.2	113.7	614.0	467.0	886.7	353.4	272.7
Eukiefferella sp.	0.2	1.6	0.2	0.3	−0.1	−1.3	0.1	4.0	0.3	2.7	0.3	−1.2	0.4	2.8	0.4	1.5	0.0	−1.3
Glossosoma sp.	11.8	194.8	17.6	218.5	5.8	23.7	0.0	419.4	2.8	87.3	2.8	−332.1	3.3	134.0	6.7	327.2	3.3	193.3
Neophylax sp.	0.0	7.6	166.1	27.7	166.1	20.1	0.0	16.6	13.5	54.9	13.5	38.3	0.0	12.4	0.0	26.7	0.0	14.3
Total	121.3	902.1	362.7	868.5	241.4	−33.7	0.1	854.7	105.7	437.5	105.6	−417.2	117.4	763.1	474.1	1242.1	356.7	479.0
Predator																		
Ceratopogonidae	11.4	7.1	23.1	18.2	11.7	11.1	3.6	2.8	3.7	0.4	0.1	−2.4	5.0	1.9	7.4	2.8	2.3	0.9
Dicranota sp.	110.6	29.6	75.9	46.5	−34.7	16.9	38.7	20.4	60.8	13.1	22.1	−7.3	154.4	17.7	119.3	40.7	−35.1	23.0
Hexatoma sp.	3.6	0.5	1.8	−0.1	−1.7	−0.6	3.9	1.1	3.4	0.4	−0.5	−0.7	3.8	2.6	5.2	0.0	1.4	−2.6
Isogenoides sp.	74.5	80.3	51.9	31.0	−22.5	−49.4	233.6	190.1	109.9	35.5	−123.7	−154.5	40.4	33.5	33.4	38.8	−6.9	5.4
Rhyacophila sp.	219.1	151.7	201.7	59.8	−17.5	−91.9	159.1	50.1	42.6	25.7	−116.5	−24.4	35.1	39.9	97.9	27.8	62.9	−12.0
Total	419.2	269.1	354.4	155.4	−64.7	−113.8	438.9	264.4	220.4	75.0	−218.5	−189.4	238.6	95.6	263.2	110.2	24.6	14.6
Community total	1460.6	1852.8	1278.4	1198.9	−182.3	−653.9	888.9	1256.5	756.9	593.5	−131.9	−663.0	543.3	1014.5	972.1	1402.1	428.7	387.7

**Indicates a significant change in mean macroinvertebrate $\delta^{13}C$ value after wood addition. D is the difference in treatment (T) and control (C). B is before (Y0) and A. is after (Y1 and Y2) wood addition. Functional groups are in bold.*

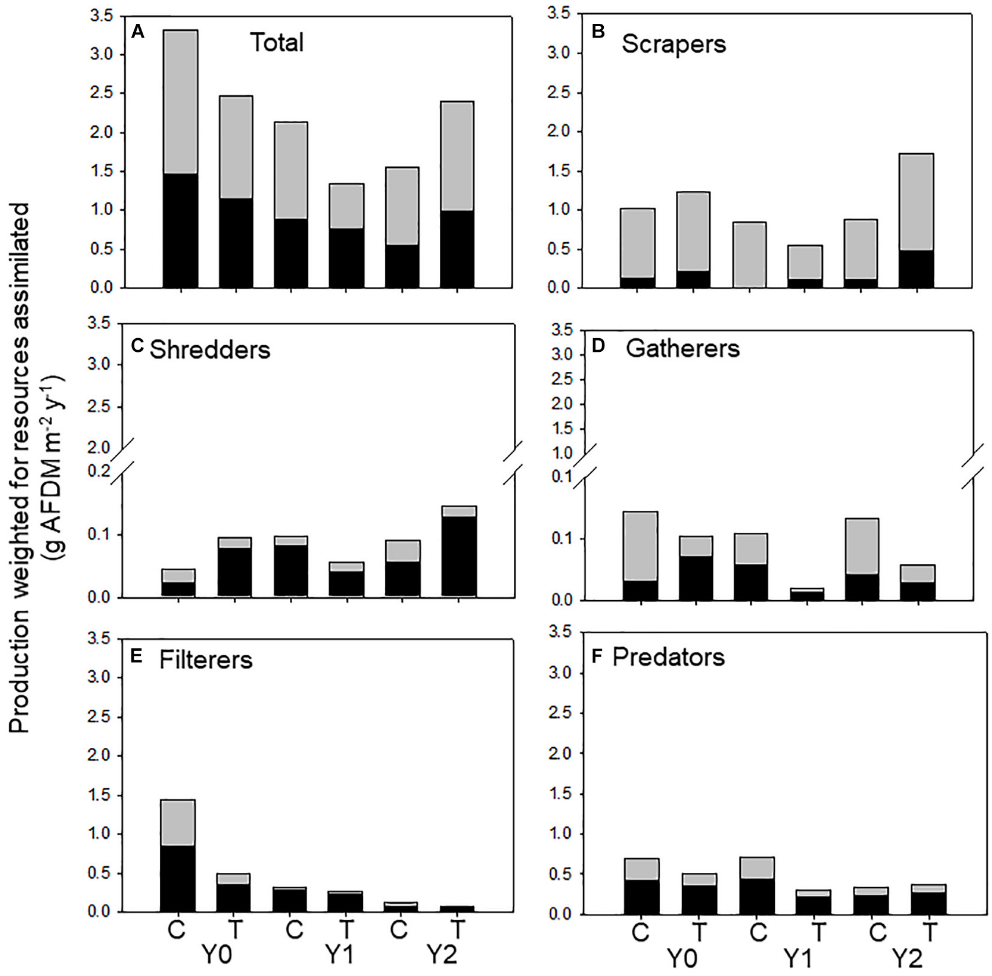

FIGURE 4 | Total macroinvertebrate secondary production supported by allochthonous (dark bars) and autochthonous (light bars) food resources assimilated by macroinvertebrates (categorized by functional feeding groups) in an upstream control C and downstream wood-added reach (treatment, T) 1 year before (Y0) and 1 (Y1) and 2 years (Y2) after wood addition. **(A)** Is total macroinvertebrate production weighted for resource assimilation, **(B)** is scraper production weighted for resource assimilation, **(C)** is shredder production weighted for resource assimilation, **(D)** is gatherer production weighted for resource assimilation, **(E)** is filterer production weighted for resource assimilation, and **(F)** is predator production weighted for resource assimilation.

DISCUSSION

Implications for Management and Restoration

Combining $\delta^{13}C$ signatures to get allochthonous and autochthonous C assimilation with secondary production provided a unique investigation into the macroinvertebrate trophic response to wood addition (**Figure 6**). The combined approach of C assimilation and macroinvertebrate secondary production indicated changes in the consumption and flow of allochthonous and autochthonous C through the macroinvertebrate food web. Here we demonstrated that the abundance of organic matter and locally exposed cobble and gravel, which supports periphyton, could drive increased allochthonous and autochthonous C flow to several macroinvertebrate functional feeding groups most notably greater autochthonous C contribution to scrapers and greater allochthonous C contribution to filterers shredders (**Figure 3**). Taxon-specific seasonal and annual shifts in C assimilation were documented (**Supplementary Appendix 1**). Still, we observed changes in total resource consumption from the coupled increase in organic matter standing crop and substrate sorting at a local scale (i.e., around the added logs) following wood addition (Entrekin et al., 2008). Unfortunately, we did not replicate this study, which is a commonly encountered challenge for reach-scale quantitative food web approaches that measure energy flow (e.g., Cross et al., 2007). Despite the limits for generalizations, our results support methods for future evaluations of restoration (see also Vander Zanden et al., 2006) by stressing the need for trophic-based analysis to assess restoration success or failure in terms of resource availability and energy flow. The combined structural and functional approach here points to physical changes from the restoration that increased allochthonous and autochthonous C flow (**Figure 5**) and increased food web

TABLE 3 | Total amount of allocthonous (alloch) and autochthonous (auto) food sources consumed by each taxon (mg AFDM m^{-2} y^{-1}).

	Y0						**Y1**						**Y2**					
	C		***T***		***D***		***C***		***T***		***D***		***C***		***T***		***D***	
Taxon	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**	**Alloch**	**Auto**
Shredder																		
Amphinemura sp.	118	111	71	31	−47	−81	19	43	63	38	44	−5	88	77	50	20	−38	−57
Capniidae	292	32	91	8	−201	−24	818	0	612	13	−205	13	469	135	890	97	422	−39
Nemoura sp.	0	43	1340	122	1340	78	0	70	176	83	176	13	126	95	57	37	−69	−58
Tipula sp.	145	0	425	0	280	0	1233	0	142	0	−1091	0	697	0	2206	0	1509	0
Total	554	187	1927	160	1373	−27	2070	113	994	134	−1076	21	1380	307	3203	153	1824	−154
Gatherer																		
Diamesinae	76	403	283	135	207	−268	321	104	0	0	−321	−104	97	105	177	55	80	−50
Ephemerella sp.	149	49	304	30	156	−19	621	442	1434	194	813	−249	41	39	183	27	141	−12
Hesperoconopa sp.	577	46	1923	44	1346	−2	223	41	2088	100	1865	60	638	125	503	98	−135	−26
Oligochaeta	317	0	85	3	−233	3	514	22	214	2	−300	−20	391	7	431	39	40	32
Paramethocnemus sp.	10	0	6	0	−4	0	11	1	8	0	−4	−1	10	0	7	0	−3	0
Tanytarsus sp.	305	58	593	2	288	−56	406	71	172	0	−234	−71	409	252	175	53	−234	−199
Total	1434	557	3194	214	1760	−342	2095	681	3915	296	1820	−385	1585	528	1475	272	−110	−255
Filterer																		
Dolophilodes sp.	1279	186	595	14	−684	−171	0	0	0	0	0	0	101	16	795	26	694	10
Parapsyche sp.	2601	257	8316	248	5714	−9	2000	223	5058	133	3058	−90	1471	432	700	30	−771	−403
Prosimulium sp.	11528	2450	0	823	−11528	−1627	736	128	803	111	67	−17	148	16	58	17	−90	1
Simulium sp.	5610	2044	0	0	−5610	−2044	4346	0	0	0	−4346	0	0	0	0	0	0	0
Total	21017	4936	8910	1085	−12107	−3852	7082	351	5861	244	−1221	−106	1719	464	1552	73	−167	−392
Scraper																		
Baetis spp.	2731	5818	4469	5183	1738	−635	0	3457	2228	2439	2228	−1018	2841	5117	11676	7389	8834	2272
Eukieffehella sp.	5	13	4	3	−1	−11	2	33	8	23	6	−10	10	23	10	12	0	−11
Glossosoma sp.	296	1623	441	1821	145	198	0	3495	70	727	70	−2768	83	1117	167	2727	84	1611
Neophylax sp.	0	63	4154	231	4154	167	0	138	338	457	338	319	0	103	0	222	0	119
Total	3032	7518	9067	7237	6035	−281	2	7123	2643	3646	2641	−3477	2935	6359	11852	10351	8918	3991
Predator																		
Ceratopogonidae	285	59	577	152	292	93	91	23	93	3	3	−20	126	16	185	23	59	7
Dicranota sp.	2766	246	1898	387	−868	141	968	170	1519	109	552	−61	3860	148	2982	339	−878	192
Hexatoma sp.	89	4	46	−1	−43	−5	97	9	84	3	−13	−6	94	21	129	0	35	−21
Isogenoides sp.	1862	669	1299	258	−564	−411	5840	1584	2749	296	−3092	−1288	1009	279	836	324	−173	45
Rhyacophila sp.	5478	1264	5042	499	−436	−765	3978	417	1066	214	−2912	−204	876	332	2448	232	1572	−100
Total	10479	2243	8861	1295	−1619	−948	10973	2203	5511	625	−5462	−1578	5965	796	6579	918	614	122
Community total	36516	15440	31959	9991	−4557	−5449	22221	10471	18923	4945	−3298	−5525	13584	8454	24661	11766	11078	3312

**Indicates a significant change in mean macroinvertebrate δ^{13}C value after wood addition. D is the difference in treatment (T) and control (C). B is before (Y0) and A. is after (Y1 and Y2) wood addition. Functional groups are in bold.*

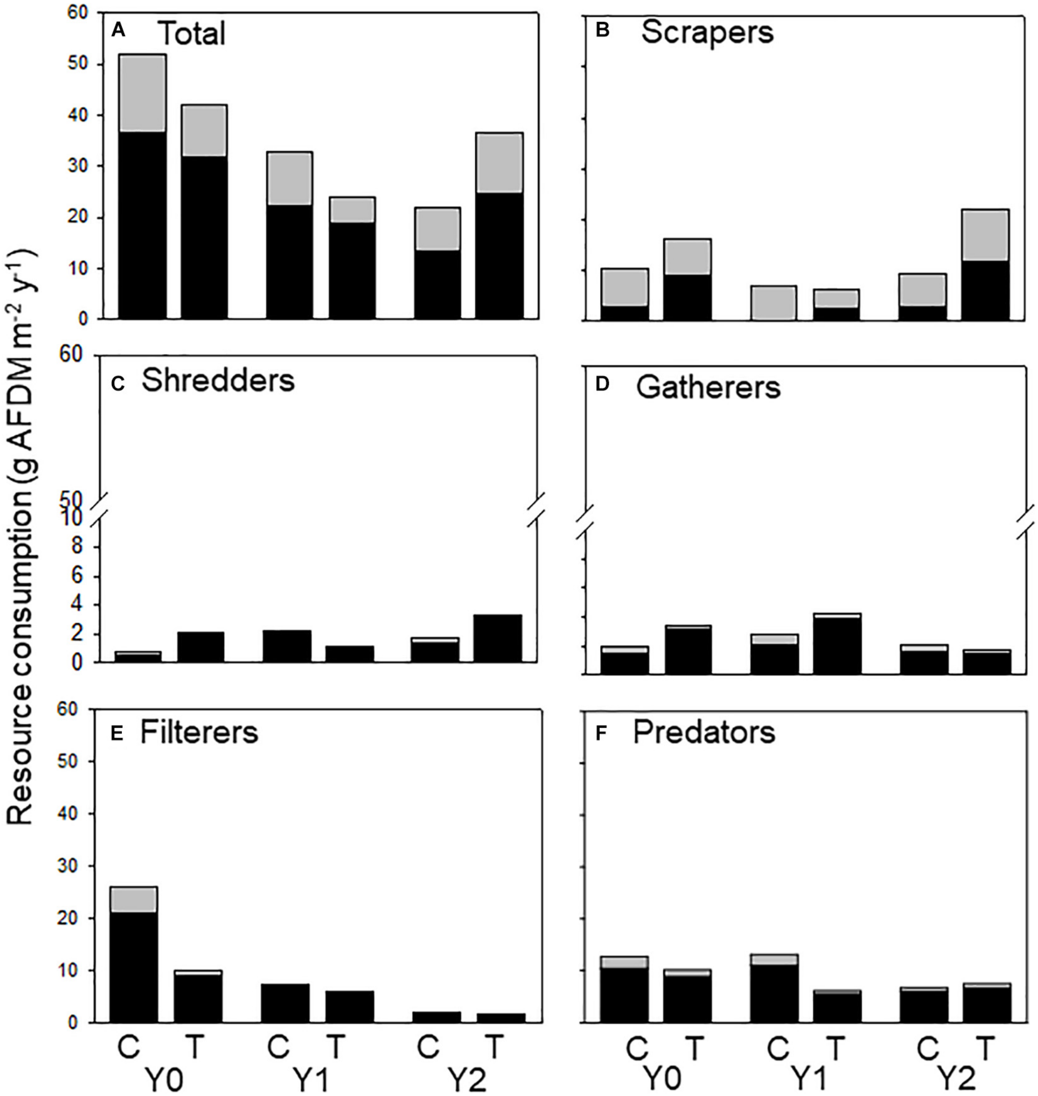

FIGURE 5 | Allochthonous (dark bars) and autochthonous (light bars) C consumed by macroinvertebrates (categorized by functional feeding groups) in an upstream control C and downstream wood-added reach (treatment, T) 1 year before (Y0) and 1 (Y1) and 2 years (Y2) after wood addition. **(A)** Represents all macroinvertebrate consumption, **(B)** represents scraper consumption, **(C)** represents shredder consumption, **(D)** represents gatherer consumption, **(E)** represents filterer consumption and **(F)** represents predator consumption of allochthonous and authochonous carbon.

connections predicted from large wood additions in temperate streams (**Figure 6**).

Modest changes in C flow following wood additions were from a combination of greater assimilation of allochthonous and autochthonous C by a few macroinvertebrate taxa. *Baetis* mayflies, a multivoltine taxa, responded with greater secondary production that was fueled by increasing amounts of allochthonous C. *Glossosoma* caddisflies also showed an increase in production fueled by autochthonous C (**Figure 6**). Allochthonous and autochthonous resources were nearly equally important in this forested temperate stream. Typically, allochthonous resources are most important and organic matter standing crop predicts macroinvertebrate secondary production (Wallace et al., 1987, 2015); however, in the main channel of our stream, autochthony was as well documented. For example, 2 years after the wood additions, ~40–50% of the macroinvertebrate community was supported by autochthonous C (**Figure 3**). In contrast to a manipulation of allochthonous C in an Appalachian stream (Wallace et al., 1997b), autochthonous C was a more important and substantial resource to the food web (**Figures 4**, **5**). The difference in our study compared to studies in the Appalachian stream is the absence of a dense understory of rhododendron and a mature forest (Greenwood and Rosemond, 2005; Wallace et al., 2015). In the Appalachian streams, rhododendron and a more mature forest canopy limited light for primary production (Greenwood and Rosemond, 2005). Light was not limiting during seasons of open canopy in our study stream and this was likely the primary reason for

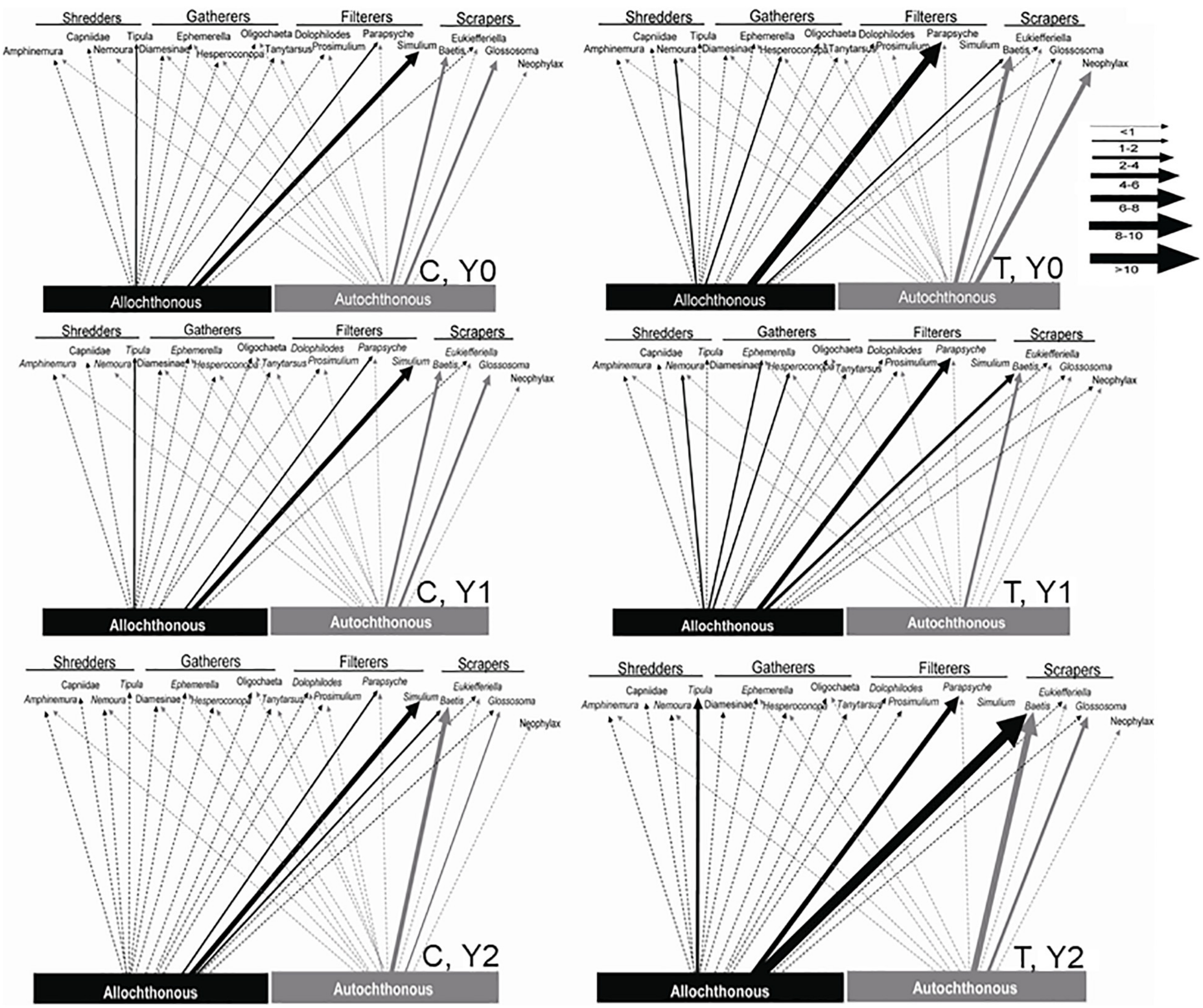

FIGURE 6 | Allochthonous (dark lines) and autochthonous (light lines) C flow to macroinvertebrate taxa in an upstream control C and downstream wood-added reach (treatment, T) 1 year before (Y0) and 1 (Y1) and 2 years (Y2) after wood addition. Arrow widths represent the proportional importance of allochthonous and autochthonous C.

greater autochthonous resource availability in this study stream compared to others conducted in headwater streams draining in-tact forests (Hoellein et al., 2007). Still, allochthonous C was the primary C source contributing to macroinvertebrate secondary production, particularly for shredders (∼50–80% contributed to secondary production), gatherers (∼60–90%), filterers (∼55–90%), and predators (∼60–80%) (**Figure 3**). The overall contribution of allochthonous C to secondary production ranged from 50 to 60% because scraper production was relatively high compared to the other functional groups (**Figure 4**).

Seasonal Effects of Added Wood on Macroinvertebrate δ^{13}C

While annual average macroinvertebrate isotopic signatures changed only a little through space and time, taxon-specific signatures did change seasonally (**Figure 1**). Taxa with seasonally variable δ^{13}C signatures may act as ‘indicators’, useful in evaluating ecosystem restorations aimed at changing or increasing basal resources [sensu 44]. In this study, *Glossosoma*, considered obligate consumers of algae, showed little variability in δ^{13}C across months, years, and stream reaches; therefore, their production rather than their δ^{13}C signatures may serve as a metric of restoration success (**Table 1** and **Supplementary Appendix 1**). In contrast, facultative feeders such as the shredding stonefly *Nemoura* sp., and *Baetis* spp., showed spatially and temporally variable δ^{13}C values, and so their average δ^{13}C values at a population level may be more indicative of seasonal changes in basal food resource availability. Using the natural abundance of C stable isotopes for assessing resource acquisition complements assessments based on community-level diversity and tolerance values (e.g., Gratton and Denno, 2006).

Influence of Large Dead Wood on Macroinvertebrate C Flow

Given the forested nature of our study stream and the well-documented role of leaves and wood in governing macroinvertebrate secondary production, we hypothesized that most C in stream macroinvertebrates would bear an allochthonous signature (Wallace et al., 2015). Indeed, we predicted primary production would be limited by the deciduous canopy that was 75% closed in the winter and spring and 90% closed in the summer and autumn (Entrekin et al., 2008). Furthermore, a comparison of organic matter standing crop across studies and years show a strong positive relationship with macroinvertebrate secondary production suggesting reliance on allochthonous C as a primary food resource (e.g., Newbold et al., 1997; Webster et al., 1997; Hall et al., 2001; Chadwick and Huryn, 2007; Cross et al., 2007; Entrekin et al., 2007). However, these studies did not measure assimilation and algae can contribute more to production than often assumed from feeding mode analysis and correlations to resource availability (Finlay, 2001; Hall et al., 2001; Marcarelli et al., 2011). Therefore, we were surprised to find that the autochthonous C supported 47–59% of secondary macroinvertebrate production in both reaches (**Figure 3**).

The few studies that measured the contribution of primary production to macroinvertebrates in temperate headwater streams have based their conclusions on gut content analysis or tracer stable isotopes. For example, Mayer and Likens (1987) used gut contents to conclude that algae were more important than expected for caddisflies in forested headwater streams. More recently, allochthonous and autochthonous C resource contributions have been delineated for entire macroinvertebrate communities in a variety of biomes using stable isotopes with the consensus being algae were more important to the food web than expected (Guo et al., 2016; Brett et al., 2017; Neres-Lima et al., 2017). In fact, macroinvertebrates sampled from headwaters in the Salmon River, where the River Continuum Concept was developed, showed a surprising amount of diatoms in their guts (Rosi-Marshall et al., 2016). Still, macroinvertebrates in the Salmon River headwaters consumed more allochthonous C. In fact, macroinvertebrates also consumed about 80% allochthonous C in this study. These two perspectives are important to consider: consumption versus assimilation. Both processes are essential aspects of C cycling; however, each indicate different C fates. Carbon assimilation compared with available resources can be used to quantify trophic transfer efficiency, while assimilation subtracted from consumption quantifies egestion that is a measure of C recycling (i.e., carbon available in a different form to a range of organisms). Therefore, autochthonous C trophic transfer was relatively high compared to resource availability. Yet, allochthonous C consumption was relatively high and critical for C recycling. Lamberti and Steinman (1997) reviewed studies from 30 streams, mostly in the Northern Hemisphere, that indicated periphyton was an energetically important food resource in a range of stream sizes, biomes, and geographic areas. Furthermore, Finlay (2001) compiled macroinvertebrate δ^{13}C values from 70 streams worldwide that indicated functional groups other than scrapers and shredders were assimilating significant amounts of epilithic algae, particularly in streams with catchments greater than 10 km^2. Our results, confirming the importance of primary producers to macroinvertebrate production reflect that (1) algae are assimilated more efficiently than leaf litter because of higher nutritive value (e.g., lower C:N:P content) (Berg and Hellenthal, 1992), (2) algae have higher turnover rates that increase availability to macroinvertebrate consumers (Lamberti et al., 1989), and (3) algae are available year round even in streams with nearly closed deciduous canopy (Hamilton et al., 2004).

When we compared the amount of algal C available (calculated as gross primary production; GPP) to the total amount of algae consumed, we found that macroinvertebrates were consuming 4–36% of the total GPP [or about 8–72% net primary production (NPP)]. This autochthonous contribution of C to the macroinvertebrates is greater than we anticipated and greater than in tropical forested streams (Neres-Lima et al., 2017; Rosas et al., 2020). The contribution of autochthonous C was likely relatively high in this stream and perhaps others because: (1) filtering taxa were likely ingesting recycled autochthonous C in the form of epilithic scrapers and sloughed particles (e.g., Strayer, 1988; Benke and Wallace, 1997), (2) scrapers were likely increasing algal turnover rates through efficient grazing, while keeping biomass low (e.g., Lamberti et al., 1995), and (3), estimation errors associated with both whole-reach estimates of GPP and NPP (e.g., Hall and Tank, 2003) and using published estimates of C assimilation by macroinvertebrates using measurements of production could under estimate C consumption (e.g., Morin et al., 1987). Our results highlight assumptions that must be made and the difficulty in estimating the contribution of autochthonous and allochthonous resources in systems with consumers ingesting a complex mixture of both types simultaneously.

When we compare the amount of leaf litter consumed by macroinvertebrate community compared to the amount available, consumption values ranged from 13 to 40% of available. Organic matter evidently was not limiting macroinvertebrate population growth on an annual scale, although it might have been limited in some seasons, as has been shown in other studies (e.g., Richardson, 1991; Dobson and Hildrew, 1992; Marks, 2019). The addition of large wood increased the percentage of GPP and coarse benthic organic matter consumed by 25 and 10%, respectively, for the macroinvertebrate community in the treatment reach relative to the control reach. Other studies have found that changes in microhabitat, such as increased leaf litter accumulations, may change the invertebrate community structure and increase production (e.g., Wallace et al., 1997a; Kobayashi and Kagaya, 2004). In our study, increased patches of inorganic coarse substrate and deposited leaf litter and fine sediment associated with the added wood seemed to have increased production by the shorter-lived taxa, such as *Baetis*, resulting in increased community-level secondary production (Entrekin et al., 2009). Longer-term increases in organic matter retention are predicted to increase the relative importance of allochthonous organic matter to the food web and sustain greater production and trophic transfer in the future.

DATA AVAILABILITY STATEMENT

All macroinvertebrate datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

SE co-designed the experiments, analyzed the data, and was the lead manuscript writer. ER, JT, GL, and TH co-designed the experiments and provided critical input and edits to the manuscript.

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SUPPLEMENTARY MATERIAL

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Drought-Induced, Punctuated Loss of Freshwater Mussels Alters Ecosystem Function Across Temporal Scales

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Punctuated, mass mortality events are increasing for many animal taxa and are often related to climatic extremes such as drought. Freshwater mussels are experiencing increased mass mortality events linked to hydrologic drought. Because mussels play important functional roles in rivers it is important to understand the ecosystem effects of these die-offs. Here, we address how mass mortality events of mussels caused by drought may impact stream ecosystem function. We first present a conceptual model, based on the literature, of how mussel mass mortality should affect different ecosystem functions across various ecological time scales, from hours to decades. Next, we highlight two case studies of drought-linked, mussel-mass mortality events from rivers in the southern U.S. We then present the results of an experiment we performed quantifying the ecosystem effects of a punctuated mussel die-off. Finally, we combine our experimental results with field data from a recent mussel die-off to predict how mussel losses will influence ecosystem function. Based on the presented case studies, our mesocosm experiment, and our extrapolated nutrient pulse due to a mussel die-off, we conclude that stream ecosystems are extensively altered following mussel mass mortality events. Mussel loss is governed by drought severity, location within the river network, and species-specific drought tolerances. In the short term, decomposing carrion from mussel die-offs releases a large pulse of nutrients into the water which stimulates food web productivity. In the long term, the overall loss of mussel biomass, and the loss of functional traits as more sensitive species decline, leads to decreases in ecosystem function which may take decades to recover. Drought and human demand for water will make mussel die-offs more likely in the future and it is unlikely that drought sensitive species will recover without changes in water management and restoration of populations through mussel propagation. Our research provides an example of how the loss of an abundant, long-lived organism has cascading, and long-term impacts on ecosystems.

Keywords: mass mortality event, resource pulse, die-off, bivalve, freshwater mussel, drought, nutrient cycling

INTRODUCTION

Resource pulses are episodes of increased resource availability in space and time that are relatively rare, of large magnitude, and usually of short duration (Yang et al., 2008). These pulses are widespread and often result from climatic and environmental factors. Resource pulses can result from the mass die-offs of animals, such as 17-year cicadas, spawning salmon, and even wildebeest, and are increasingly recognized as important components of ecosystem function (Yang et al., 2008; Subalusky et al., 2017). Mass mortality events, or die-offs, are increasing in frequency across most taxa (Fey et al., 2015), thus it is important that we understand how these events affect ecosystem function (Baruzzi et al., 2018). In freshwater systems, unionid mussels play important structural and functional roles and are also experiencing increasing mass mortality events globally (Lydeard et al., 2004; Wenger et al., 2018) that are often linked to climatic events such as drought (Vaughn et al., 2015). Thus, they are a useful system for investigating the ecosystem effects of mass mortality events, particularly as related to environmental change (Fey et al., 2015).

Here, we address how mass mortality events of unionid mussels caused by drought may impact stream ecosystem function short-term and long-term. We first present a conceptual model, based on the literature, of how mussel mass mortality should affect different ecosystem functions across various ecological time scales, from hours to decades. Next, we highlight two case studies of drought-linked, mussel-mass mortality events from rivers in the southern U.S. We then present the results of an experiment we performed quantifying the ecosystem effects of a punctuated mussel die-off. Finally, we combine our experimental results with field data from a recent mussel die-off to predict how mussel losses will influence ecosystem function.

HOW DO MUSSEL MASS MORTALITY EVENTS IMPACT ECOSYSTEM FUNCTION? A CONCEPTUAL MODEL

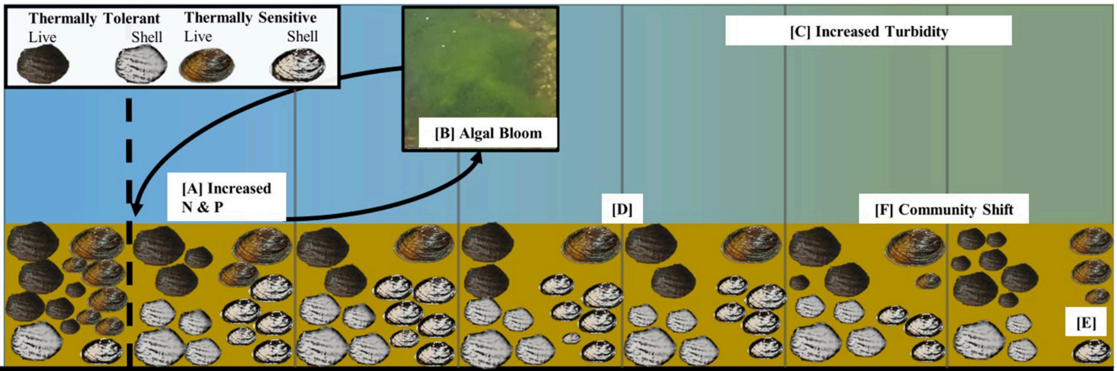
Freshwater mussels (order Unionida, hereafter mussels or unionids) are sedentary mollusks that live burrowed in stream sediments where they filter the water and transfer energy and nutrients from the water column to benthos. Nutrients excreted and biodeposited by mussels stimulate instream microbial, primary, and secondary production and are even exported to riparian areas (Allen et al., 2012; Vaughn, 2018). Mussels provide habitat for other organisms through the biogenic structure of their shells and by changing hydrodynamic conditions at the sediment-water interface (Sansom et al., 2018b). They are long-lived (6–100 years) with high native biodiversity in eastern North America (Williams et al., 1993), often live in high density, multi-species aggregations (hereafter mussel beds) that can persist in rivers for many decades and can make up most of the invertebrate biomass in many perennial rivers (Sansom et al., 2018a). Mass mortality of mussels has been linked to increasing drought, either from emersion (Atkinson et al., 2014) or from low dissolved oxygen and high temperatures associated with decreased water volume (Gagnon et al., 2004), as drying in streams is often accompanied by increased water temperatures and diel oxygen shifts (Mosley, 2015). How mussels respond to drought conditions depends on individual species' physiological tolerances, drought severity, and abiotic conditions (Gagnon et al., 2004; Golladay et al., 2004; Haag and Warren, 2008; Gough et al., 2012). When mass mortality of mussels occurs, their loss should influence stream ecosystem functions in a variety of ways across ecological time scales (**Figure 1**). We highlight these predicted effects below.

Mussel Mortality Feedback Loop

Mussel species vary in their physiological tolerance and response to stress (Spooner and Vaughn, 2008). Species that are sensitive to low oxygen (hypoxia sensitive) or higher temperatures (thermally sensitive) are less likely to survive during a hydrologic drought, thus thermally tolerant and/or hypoxia tolerant mussels become the dominant species within the assemblage (Gagnon et al., 2004; Atkinson et al., 2014). Mussel soft tissue can decay within seven days, as shown below. Decomposing soft tissue releases a pulse of nitrogen and phosphorus into the water column and interstitial spaces (**Figure 1A**; Cherry et al., 2005; Atkinson et al., 2014). Depending on stream discharge, this nutrient pulse moves downstream over a few hours/days. Pore water can retain high nutrient concentrations longer, potentially exposing burrowing unionids to lethal nutrient concentrations (Cooper et al., 2005; Gough et al., 2012). After the loss of mussels, shifts in algal production, and turbidity are driven by stream discharge. If the stream becomes intermittent (a series of drying pools), turbidity will likely decrease due to increased sedimentation while algal blooms will likely form in stagnant areas (Mosley, 2015). During intermittence, the combination of reduced biofiltration and the release of nutrients into the water from mussel soft tissue decay encourages large algal blooms (Gagnon et al., 2004), which leads to high respiration rates at night, further reducing dissolved oxygen concentrations and stressing the remaining mussels, leading to additional mortality (**Figure 1B**). This cycle can also exacerbate feedback among deaths within mussel beds; algal blooms cause mortality in remaining hypoxia-intolerant bivalves, worsening algal blooms, and depressing dissolved oxygen, further stressing and eventually killing hypoxia-tolerant mussels. If the stream remains perennial, turbidity will increase through the addition of suspended solids from upstream and algal blooms become less likely. Algal blooms and/or increased turbidity can persist because of a reduction in biofiltration by freshwater mussels (**Figure 1C**).

Reduced Biofiltration

Immediately after a mass mortality event, biofiltration is greatly reduced in part to both residual stress on the remaining living mussels and biomass loss from mussel mortality (Vaughn et al., 2015). Biofiltration by the remaining mussels will gradually increase within the following days, but is likely to remain low until mussel biomass is replaced, unlikely for at least a decade (**Figure 1E**). Reduced biofiltration drastically increases the time required for the remaining mussels to filter a given



	PRE MASS MORTALITY[a,b]	HOURS	DAYS	WEEKS	MONTHS	YEARS	DECADES
BIOFILTRATION	~5,200 L h^{-1} m^{-2}	Reduced	[B] Reduced biofiltration, paired with increased nutrients, lead to algal blooms[a,c,d]		[C] Potentially altered biofiltration capacity & timing, due to community change		
NUTRIENT REGENERATION	~345 µmol N m^{-2} h^{-1} ~26 µmol P m^{-2} h^{-1}	[A] Increased N & P in the water column[e] & the interstitial space[f]	Increased N & P in the interstitial space[f]			Loss of nutrient capacitance* & shift of excretion stoichiometry because of the homogenized community and reduced biomass	
			[D] Loss of nutrient capacitance, due to individuals lost[g]				
NUTRIENT STORAGE	~47 g N m^{-2} ~4.8 g P m^{-2}	Soft tissue storage reduced while shell is stable[g]			[E] Shells begin dissolving, slowly releasing nutrients into the water column[h]		Storage reduced until biomass completely rebounds
HABITAT	~28 ind. m^{-2} ~5 species m^{-2}	Increased interstitial spaces from shells without tissue			[E] Decreased habitat heterogeneity of mussels & mussel shells[i]		More homogenous benthos

FIGURE 1 | Conceptual model depicting ecosystem function shift due to unionids at short and long time scales. Pre mass mortality information based on 25°C, downstream values for 1991 in Vaughn et al. (2015) for the biofiltration and community structure data and on Atkinson and Vaughn (2015) for downstream nutrient excretion and storage information. *Loss of nutrient capacitance dependent on mussel recruitment. Time increases to the left and the impact on mussels of four main ecosystem functions is described and depicted. Capital letters refer to distinct processes in the model, while lower case letters refer to sources: (a) Vaughn et al. (2015), (b) Atkinson and Vaughn (2015), (c) McDowell et al. (2017), (d) Gagnon et al. (2004), (e) Cherry et al. (2005), (f) Cherry et al. (2005), (g) Atkinson et al. (2014), (h) Atkinson et al. (2018), (i) Ilarri et al. (2018).

amount of water, reducing material exchange between the water column and benthos (Baustian et al., 2014; Vaughn et al., 2015).

Reduced Nutrient Capacitance and Storage

Short-term nutrient storage in mussel soft tissue is greatly reduced through decomposition (Atkinson et al., 2014). As mussels filter feed, they act as "nutrient capacitors," accumulating, storing, and releasing energy and nutrients (carbon, nitrogen, and phosphorus) at different rates based on their age and species' traits (Strayer, 2014; Atkinson et al., 2018). Following mass-mortality, remaining mussel assemblages have lower abundance, age diversity, and species diversity, reducing their ability to filter seston and excrete nutrients. This reduced nutrient capacitance may result in longer nutrient spirals and more downstream transport of nutrients, likely due to an increase in nutrient uptake length (**Figure 1D**; Atkinson et al., 2014). Shells of deceased mussels lose ~50% mass by 15 years through mechanical and chemical dissolution, which reduces the nutrient storage and shell habitat within the mussel bed as particle size becomes more homogeneous (**Figure 1E**; Atkinson et al., 2018).

Changes in Habitat Provided by Shells

Live mussels and their spent shells physically modify the environment in streams, providing unique habitat for other organisms. Tissue decay potentially creates interstitial spaces within the substrate, which can be used by both macroinvertebrates and fish. Shells vary in shape and size across species and age and can accumulate in the sediment at different rates; thus, shell habitat can harbor variable macroinvertebrate communities depending on the shells species of origin (Bódis et al., 2014). While shells represent hard surfaces for macroinvertebrates, they dissolve over time. Shell dissolution is fastest in flowing waters with low calcium carbonate concentrations and thin, small shells dissolve faster than thicker, larger shells (Strayer and Malcom, 2007; Ilarri et al., 2015, 2019). While bivalve soft tissue decomposes quickly, shells persist for many decades (5–30 years), providing habitat for other stream biota (Strayer and Malcom, 2007; Ilarri et al., 2015; Atkinson et al., 2018). Over time, the benthos will be more homogenous as old shells dissolve and new relic shells are produced by a less diverse mussel assemblage ultimately altering benthic microhabitat characteristics and macroinvertebrate community structure (**Figure 1E**; Ilarri et al., 2018).

Shifts in Community Composition and Ecosystem Function

During droughts, species sensitive to low oxygen (hypoxia sensitive) or higher temperatures (thermally sensitive) face greater risk of mass mortality leading to differential survival resulting in tolerant species becoming dominant within an assemblage, changing community structure (Gagnon et al., 2004; Atkinson et al., 2014). Surviving mussels may contribute to population recovery if conditions are suitable for reproduction. Most mussels have an ectoparasitic larval phase that requires a host fish (Barnhart et al., 2008). Drought concentrates fish into drying pools as they to attempt to escape harsh conditions or die due to increased biotic and abiotic stressors (Matthews and Marsh-Matthews, 2003; Lennox et al., 2019). While mussel reproduction is unlikely limited by host density (Haag and Stoeckel, 2015), different mussel species exhibit different host specificity and infection phenology (Barnhart et al., 2008). Thus, predicting the recruitment success is difficult due to unionid's unique life histories. As it takes mussels anywhere from 9 months to 10 years to reach sexual maturity, with most mussels reaching maturity around 4 years old (Haag, 2012), in die-off affected areas, biomass is unlikely to rebound for at least a decade. Future mussel assemblage structure is dependent on the surviving mussel assemblage, the surviving fish assemblage, and the recurrence frequency of droughts. If drought frequency decreases or remains constant, the mussel community could return to its former, pre-drought structure if no mussel species were extirpated from the river basin. If droughts increase in frequency and severity as projected in many regions (Palmer et al., 2008; USGCRP., 2017), we anticipate the mussel community will become dominated by tolerant mussel species (**Figure 1E**).

Mussel species with different temperature tolerances have different, temperature-dependent biofiltration and nutrient excretion rates. Thus, when the proportion of thermally sensitive vs. tolerant species in a mussel assemblage changes, this can impact ecosystem function (Spooner and Vaughn, 2008; Vaughn et al., 2015; Atkinson et al., 2018). For example, in rivers in southern Oklahoma, *Actinonaias ligamentina* is a thermally sensitive species with higher filtration and nutrient excretion rates at summer temperatures than other mussels in the assemblage. Because of its temperature intolerance, it also has a higher mortality rate during drought than other species. Thus, when this species is lost, the overall biofiltration (**Figure 1C**) and nutrient recycling capacity (**Figure 1F**) of the community is decreased for an extended time period, even if the biomass of other species remains stable (Vaughn et al., 2015). Further, mussel species excrete at different N:P ratios, and losses can also lead to shifts in excretion stoichiometry (**Figure 1F**; Atkinson et al., 2018). These changes can cascade through the food web impacting algal, macroinvertebrate (Novais et al., 2017), and even fish dynamics.

TWO CASE STUDIES OF DROUGHT-DRIVEN MUSSEL LOSSES IN THE SOUTHERN UNITED STATES

Drought-induced mussel mass mortality events have been documented for two diverse, well-studied river systems in the southern U.S., the Lower Flint River in Georgia and the Kiamichi-Little River system in Oklahoma (**Figure 2**). These case-studies allow for a deeper understanding of how drought affects mussel assemblages, the subsequent changes in stream ecosystems, and the potential recovery time for mussel assemblages.

Lower Flint River, Georgia

The lower Flint River of southwestern Georgia experienced an extended period of below normal rainfall from 1999 until 2013. This period included three multiyear droughts that were classified as severe/exceptional. The summer of 2000 was particularly disastrous for unionids as streams in the region experienced unprecedented low flows and transitioned from perennial to intermittent. Forty-six historically-species-rich sites in lower Flint tributaries were surveyed in 1999 prior to drought onset, and a subset of these were resampled in 2000 (Gagnon et al., 2004). Stream drying had not been previously observed but became common during subsequent growing seasons (Rugel et al., 2012). Stream flow was essential for maintaining dissolved oxygen concentrations within the tolerances of freshwater mussels; dissolved oxygen between 5 and 3 mg L^{-1} resulted in 24% mortality and when dissolved oxygen fell below 3 mg L^{-1} up to 76% of mussels died (Gagnon et al., 2004; Golladay et al., 2004). These degraded physicochemical conditions differentially impacted species within the mussel communities. Riffle specialists in medium-sized streams suffered the highest mortality, while drought-tolerant, small stream species and species in larger tributaries whose habitat was buffered from drought conditions fared better (Gagnon et al., 2004). In medium-sized streams, community composition shifted toward more generalist species from riffle specialists (Gagnon et al., 2004).

The lower Flint River has been surveyed since this drought. Through 2013, there was little evidence of recovery from mortality associated with the 1999–2001 drought (Smith et al., 2015). During most summers since the initial drought, conditions were stressful, likely preventing the reproduction of surviving unionids. Reproduction of mussel populations, as evident from observation of juvenile mussels, was not apparent until rainfall approached average levels (2013–2015) (Smith et al., 2015). The extended period of below normal rainfall (1999–2013) and subsequent below normal stream flows were likely exacerbated by anthropogenic water withdrawal; the mid reaches of tributaries of the lower Flint cross the Dougherty Plain physiographic district, which is a recharge area for a heavily developed agricultural water source (Golladay et al., 2004). This case study provides evidence that mussel biomass might not recover from mass mortality events for over a decade and that anthropogenic and climate alterations can alter stream benthic communities for extended periods.

Kiamichi and Little Rivers, Oklahoma

The Kiamichi and Little Rivers in southeastern Oklahoma are adjacent, major tributaries to the Red River. This region experienced a period of exceptional drought during 2011–12 where the Kiamichi River experienced 84 days of no flow (defined

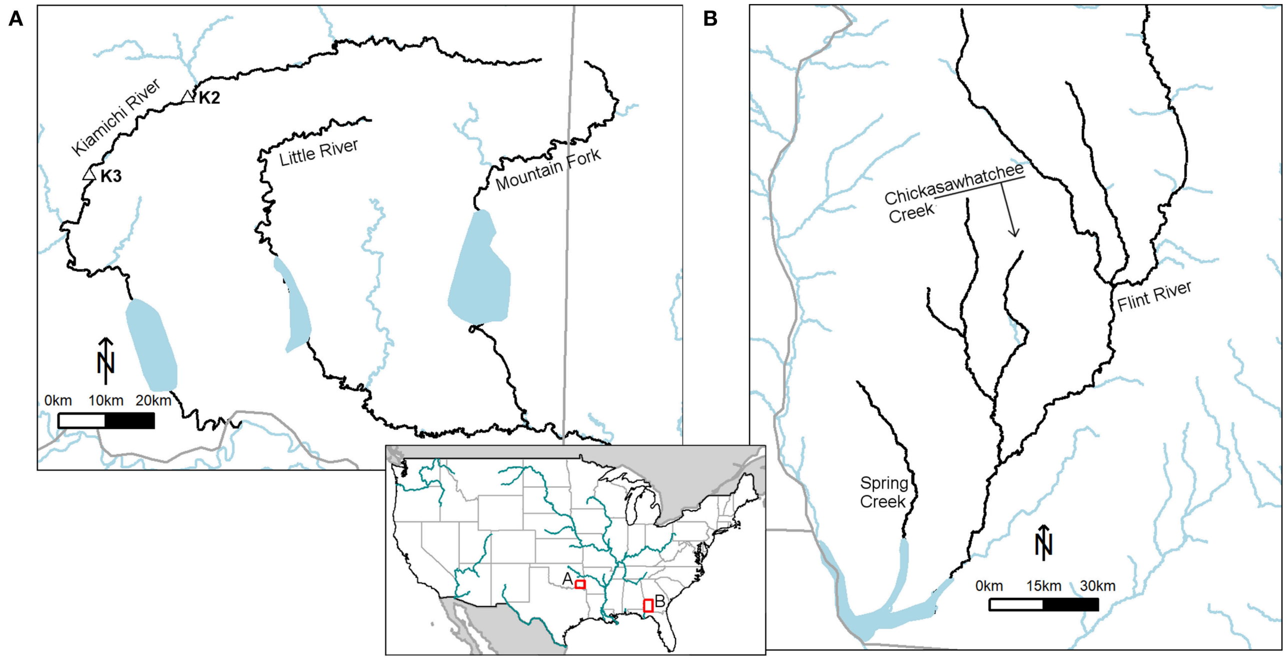


FIGURE 2 | Map of case study locations within the southern continental USA. In the bottom panel, large streams (stream order ≥8) are depicted in light blue to provide context while the focal river rivers are found within the red boxes. The letters next to the river basins indicate which panel corresponds to those river basins. Southeastern Oklahoma **(A)** contains three rivers: the Kiamichi, Little, and the Mountain Fork. K2 and K3 are sites on the Kiamichi discussed in the 'short and long-term nutrient releases following bivalve mortality' section of this manuscript. The lower Flint River **(B)** is in southwestern Georgia; rivers discussed in the case study section are named. Code from the R package *hydroMap* (DeCicoo and Blodgett, 2017).

as discharge < 0.01 m^3 s^{-1}) and 36 weeks of extreme low flow, defined as flows below the 10th percentile of flow frequency (Atkinson et al., 2014). The Little River, and its major tributary the Mountain Fork River, experienced 39 and 40 weeks of extreme low flow, respectively (Atkinson et al., 2014).

These severe drought conditions led to a mass mortality event as mussels became isolated in shallow drying pools or emersed. Mussel losses and their effects on ecosystem function were documented in two related studies. Atkinson et al. (2014) sampled mussels at three sites before a drought (2010) and at the end of the drought (2012) and assessed changes in mussel abundance and mussel-provided nutrient cycling and storage. Sixty percent of unionids died during the drought, but thermally sensitive species had a higher mortality rate, resulting in a community shift toward more thermally tolerant species. In the second study, Vaughn et al. (2015) compared mussel biomass and ecosystem services (biofiltration, nutrient cycling, and nutrient storage) at four sites in the Kiamichi River across several decades (1991, 2004, 2011). 1991 was a wet period and 2004 and 2011 were drought periods with significant mussel losses. They found that mussel biomass decreased over 60% across these sites and that ecosystem function losses mirrored the biomass losses. The sites experienced mussel biomass losses of ∼28% and corresponding declines in nitrogen recycling (22%), phosphorus recycling (15%), and ∼30% declines in areal storage of nitrogen and phosphorus (Vaughn et al., 2015).

AN EXPERIMENT QUANTIFYING THE EFFECTS OF A MUSSEL DIE-OFF ON ECOSYSTEM FUNCTION

While case studies have documented how native mussel communities change after mass mortality events, no studies have experimentally demonstrated how these losses impact ecosystem function. With our conceptual model and case studies in mind, we designed an experiment to measure ecosystem function changes that occur following a punctuated mussel mass mortality event. We conducted a mesocosm experiment at the University of Oklahoma Biological Station in the summer of 2018 where we induced mussel mortality and measured effects on ecosystem structure (water column nutrient concentrations and algal abundance) and ecosystem function (decomposition rates and ecosystem metabolism) over time. We predicted that decaying mussel tissue would increase nutrient concentrations, which would stimulate both algal growth and microbial respiration and decomposition.

Mesocosm Experiment Methods

We used 18, 1.52 m diameter, 946 L circular tanks to simulate drying stream pools. Each mesocosm (tank) was lined with ∼15 cm of gravel (1:1 ratio of 10 and 38 mm diameter gravel). We had 9 control mesocosms with no mussels and 9 mesocosms containing 31 mussel individuals, to replicate a natural mussel community in the region. Each mussel treatment mesocosm contained 13 *Actinonaias ligamentina*, 9 *Cyclonaias pustulosa*, 5 *Amblema plicata*, two *Tritogonia verrucosa*, one *Lampsilis cardium*, and one *Plectomerus dombeyanus*. This represented a low, but natural density of mussels (11.9 mussels/m^2) and reflected the freshwater mussel community of the upper Kiamichi River (Atkinson et al., 2012).

We describe sampling events and present our results relative to the day of the mussel mass mortality event: negative values indicate days before and positive values days after the mussels died. We filled mesocosms with water 12 days (day−45; see **Supplementary Table 1** for sampling dates and measurements) before adding mussels to allow the mesocosms to be naturally colonized by algae and macroinvertebrates. On day−33, we added mussels. On day−14, we introduced 10 largemouth bass (*Micropterus salmoides*; mean standard length = 95 mm, SD = 16 mm) to simulate how fish are concentrated in drying pools during early periods of drought. We removed the fish on day−2 to simulate their movement downstream during the drought as drying pools became too stressful for them (Magoulick and Kobza, 2003). Fish may have impacted water column nutrients on day−3 but had little impact on mesocosm nutrient concentrations and algal abundance (see Results below). We induced a punctuated mass mortality event on 2 July 2018 (day 0) by sacrificing the mussels in 5 of the 9 mussel mesocosms, while maintaining the 9 non-mussel controls. To produce the carrion for this stage of this experiment, we cut the adductor muscles of 155 mussels. We returned the mussel carrion to the mesocosms to allow for natural decomposition of soft tissue.

We sampled mesocosms 3 times before and 4 times after the mass mortality event, resulting in the following sampling days:−20,−15,−3, 4, 11, 25, and 39. On each sampling day we measured dissolved oxygen (DO), conductivity, and water temperature at midday (**Supplementary Table 2**). To measure water column phosphorus and nitrogen concentrations, we collected 20 mL filtered water samples (Grade F, 0.7 µm pore size, Sterlitech Kent, Washington) and froze them for subsequent nutrient analysis. We lost nutrient samples from day 11, thus we resampled the mesocosms on day 18. We filtered two water samples (Grade A, 1.6 µm pore size, Sterlitech Kent, Washington) from each mesocosm to quantify water column chlorophyll *a*. Filters were frozen for later chlorophyll estimation. On day−33, we placed six 7.6 cm^2 clay tiles with a 27.5 mm^2 fritted glass disc attached with silicone (LECO cover crucible AL P 1000; GE Silicone 1* All Purpose) on the substrate surface to allow algal colonization for sampling of benthic algal production. We removed two glass fritted discs on each sampling day and froze them for later estimation of benthic algal biomass.

We quantified soluble reactive phosphorus (SRP) with the colorimetric method (Murphy and Riley, 1962; Stainton et al., 1974; EPA Method 365.3) and ammonium (NH_4-N) using the phenol method (5.2.6 EPA Method 350.1; ASTM., 2012) for the filtered water samples. To measure chlorophyll *a* concentration, we cold-extracted water column (filters) and benthic (fritted discs) samples with acetone and measured the extractant spectrophotometrically with a correction for pheophyton (ASTM., 2012).

We followed Tank et al. (2017) to measure ecosystem metabolism as gross oxygen production on days 4, 11, 25, and 39. We quantified ecosystem metabolism by measuring dissolved oxygen production and respiration in light and dark cycles, respectively, on the glass fritted discs in 50 mL centrifuge tubes. We measured dissolved oxygen (HACH HQ40d multiple parameter meter, Loveland, Colorado) to estimate initial oxygen concentrations. We placed fritted disks in centrifuge tubes filled with the respective mesocosm's water and sealed the tubes. After allowing the discs to metabolize for an average of 1.75 h (*SD* = 0.23 h) in a common mesocosm, we re-measured the dissolved oxygen within the tubes. We then removed the water and repeated the filling process with the same tube/glass fritted disc pair. After filling the tubes, we immediately placed them in the dark in a common mesocosm for an average of 2.62 h (*SD* = 0.47 h). We then measured final dissolved oxygen and collected and froze the discs. Gross primary production (mg DO cm^{-1} h^{-1}) was calculated from the addition of net primary productivity (difference in DO in the light cycle) and the absolute value of the respiration (DO difference in the dark cycle).

To determine the decay rates of mussel tissue, we placed the combined shell and soft tissue of each of 5 *A. ligamentina* in fine mesh bags (pantyhose) in each mortality treatment (original weight mean = 297.8 g, range = 77.5–461.5 g). We chose *Actinonaias ligamentina* because it was the most abundant mussel species within each mesocosm, is thermally sensitive, and most likely to be lost during a drought (Atkinson et al., 2014). We weighed the bags every 12 h for 4.5 days, and then daily for 10 days until the shells were empty and the weight was stable. We calculated total tissue (including both the soft tissue and the shell) decay rates and soft tissue decay rates following Strayer and Malcom (2007). Soft tissue decay rates were determined by assuming the minimum weight measurement consisted of only shell material and subtracting that measurement from each weight measurement. To examine organic matter decomposition rates, we incubated three cotton strips (8 × 25 mm) in the bottom of each mesocosm beginning on day 7 (Tiegs et al., 2013). We removed strips on days 18, 28, and 38; these dates mirror the decomposition study by Novais et al. (2017) and reflect incubation times of 11, 21, and 32 days. Strips were preserved with 85% ethanol and later dried at 40°C. We determined the tensile strength of each cotton strip using a tensiometer (Mark 10 MG100) torn at 2 cm/min following Tiegs et al. (2013). As such, the tensile strength reflects the remaining organic matter of the original cotton strip and is reported in pounds.

All statistical analyses were conducted with R Core software version 3.5.3 (R Core Team, 2019). We used mixed linear models to test for differences among our dependent variables based on the fixed factor treatment, the fixed continuous variable sampling date, the interaction between the fixed variables, and a random intercept accounting for mesocosm. We included the mesocosm as a random intercept to account for the repeated measures over time on each replicate; this allows each mesocosm to have a different starting value and accounts for mesocosm dependency. Each model was checked visually for normality and homogeneity of variance of its residuals (Zuur et al., 2009); we $\log_{10}$ transformed water column ammonium, water column chlorophyll *a*, and benthic chlorophyll *a* to meet these assumptions. We used the function *lmer()* [from the R package *lme4* (Bates et al., 2015)] to perform all mixed models as we had different sample sizes for each treatment: 9 control mesocosms, 5 mortality mesocosms, and 4 live mussel mesocosms. We used the function *anova()* to conduct a type III ANOVA with Satterthwaithe's method and obtain *p*-values for all models as implemented in the *R* package *lmerTest* (Kuznetsova et al., 2017). We then used Tukey *post-hoc* tests to conduct multiple comparisons if the null hypothesis was rejected for each dependent variable as implemented in the package *emmeans* (Lenth, 2018).

Mesocosm Experiment Results

Mussel Decay

Actinonaias ligamentina soft tissue and shell had an average instantaneous decay rate of -0.016 day^{-1} across all mortality mesocosms. Within 7 days, most soft tissue had decayed within the bags. The average instantaneous decay rate of the soft tissue alone was -0.336 day^{-1}. We did not observe shell dissolution within the time frame of our experiment.

Nutrients

Following the mass mortality event, the mortality treatments had a large increase in ammonium. Ammonium (NH_4-N) was significantly higher in the mortality treatments compared to the control treatments ($F_{2,120} = 10.92$, $p < 0.001$; **Figure 3A**). Sampling day was also significant in predicting ammonium amount in the system ($F_{1,120} = 14.34$, $p < 0.001$). The interaction between treatment and sampling day was not statistically significant ($F_{2,120} = 1.17$, $p = 0.31$). Overall, ammonium in the water column significantly increased by 94.4% directly after the mass mortality event, while ammonium in the control mesocosm increased by 9.6%, although this was highly variable (ranged from 84.1 to -62.5%). SRP generally increased during the experiment ($F_{1,103} = 6.91$, $p < 0.01$; **Figure 3B**). While SRP was not significantly different between treatments ($F_{2,16} = 0.37$, $p = 0.70$), the interaction between treatment and sampling day was statistically significant ($F_{2,103} = 4.67$, $p < 0.02$). Between the die-off and the end of the experiment, SRP increased 38% in tanks that experienced the mussel die-off but decreased by 51% in control tanks.

Primary Production and Ecosystem Metabolism

Mortality treatments had higher gross primary production than the control mesocosms ($F_{2,30} = 4.11$, $p < 0.03$). Mortality treatments had higher gross primary production than live treatments, but there was not a statistical difference between the two groups ($t_{22} = 2.27$, $p = 0.08$). Sampling day was significant in explaining gross primary production ($F_{1,54} = 77.81$, $p < 0.001$). The interaction between the two terms was also not statistically significant ($F_{2,54} = 1.94$, $p = 0.16$). There was not a difference in water column chlorophyll *a* concentration between treatments ($F_{2,19} = 0.54$, $p = 0.59$), although water column chlorophyll did increase through time ($F_{1,105} = 12.51$, $p < 0.001$; **Figure 3C**). The interaction between the two terms was also not statistically significant ($F_{2,105} = 0.76$, $p = 0.47$). In late July and August, some control and live mesocosms experienced algal blooms, while mortality mesocosms had low water column chlorophyll *a* concentrations. Benthic chlorophyll *a* concentration was higher in mortality and live treatments than control treatments ($F_{2,19} = 9.10$, $p < 0.002$; **Figure 3D**). Sampling day did not predict benthic chlorophyll *a* concentration ($F_{1,106} = 0.59$, $p = 0.45$) and the interaction between treatment and sampling day was not significant ($F_{2,106} = 0.89$, $p = 0.41$). Note that ecosystem metabolism and benthic chlorophyll *a* concentrations are significantly correlated as they were measured from the same glass fritted discs ($R^2 = 0.28$, $p < 0.001$). While these variables are correlated, one measures ecosystem structure (biomass) and the other ecosystem function (respiration).

Organic Matter Decomposition

Higher tensile strength corresponds to a higher percentage of the original remaining cotton strip; thus, higher tension indicates less decomposition. Tensile strength of the cotton strips decreased with time ($F_{1,36} = 4.95$, $p < 0.04$) and was significantly different between treatments ($F_{2,18} = 5.60$, $p < 0.02$; **Figure 4**). The mean tensile strength of an unincubated cotton strip is 65.6 lbs ($SD = 2.0$ lbs).

Below, we apply these results to an actual mussel die-off to extrapolate how mussel die-offs can impact nutrient cycling in river reaches following a mass mortality event.

SCALING UP: SHORT AND LONG-TERM NUTRIENT RELEASES FOLLOWING A MASS MORTALITY EVENT

As described above, an extreme drought in the Kiamichi River in 2011 led to massive mussel mortality. We combined quantitative data on these mussel losses with nutrient release data from our mesocosm experiment to extrapolate how mussel losses impact short and long-term nutrient cycling and storage for several river reaches.

On July 30 and 31, 2011, we (CLA and CCV) sampled mussels at three sites (K2, K3 riffle, and K3 pool) severely impacted by the drought. The K2 and K3 pools were isolated pools that still contained water but were very shallow (<10 cm) and warm (K3 exceeded 40°C) (Vaughn et al., 2015). K3 contained a riffle that was completely dry. For K2 and K3 pools, we measured mussel abundance and composition by sampling ten, 0.25 m^2 quadrats following Vaughn et al. (1997); we identified and measured the length of both dead and live individuals. We returned live individuals to the mussel bed. For the K3 dry riffle we laid out 10 transects across the dry mussel bed and identified shells from 14 quadrats across each transect. We used species-specific, length-soft tissue dry mass regression equations (Hopper et al., 2018) to calculate the soft tissue mass of each mussel. We calculated areal nutrient pulses (μg L^{-1} m^{-2}) released to the river as a consequence of mussels dying as the product of nutrients released by decomposing mussel tissue to the water column in the mesocosm at sampling day 4 (μg L^{-1} g^{-1}) by the biomass loss of mussels at a site on an

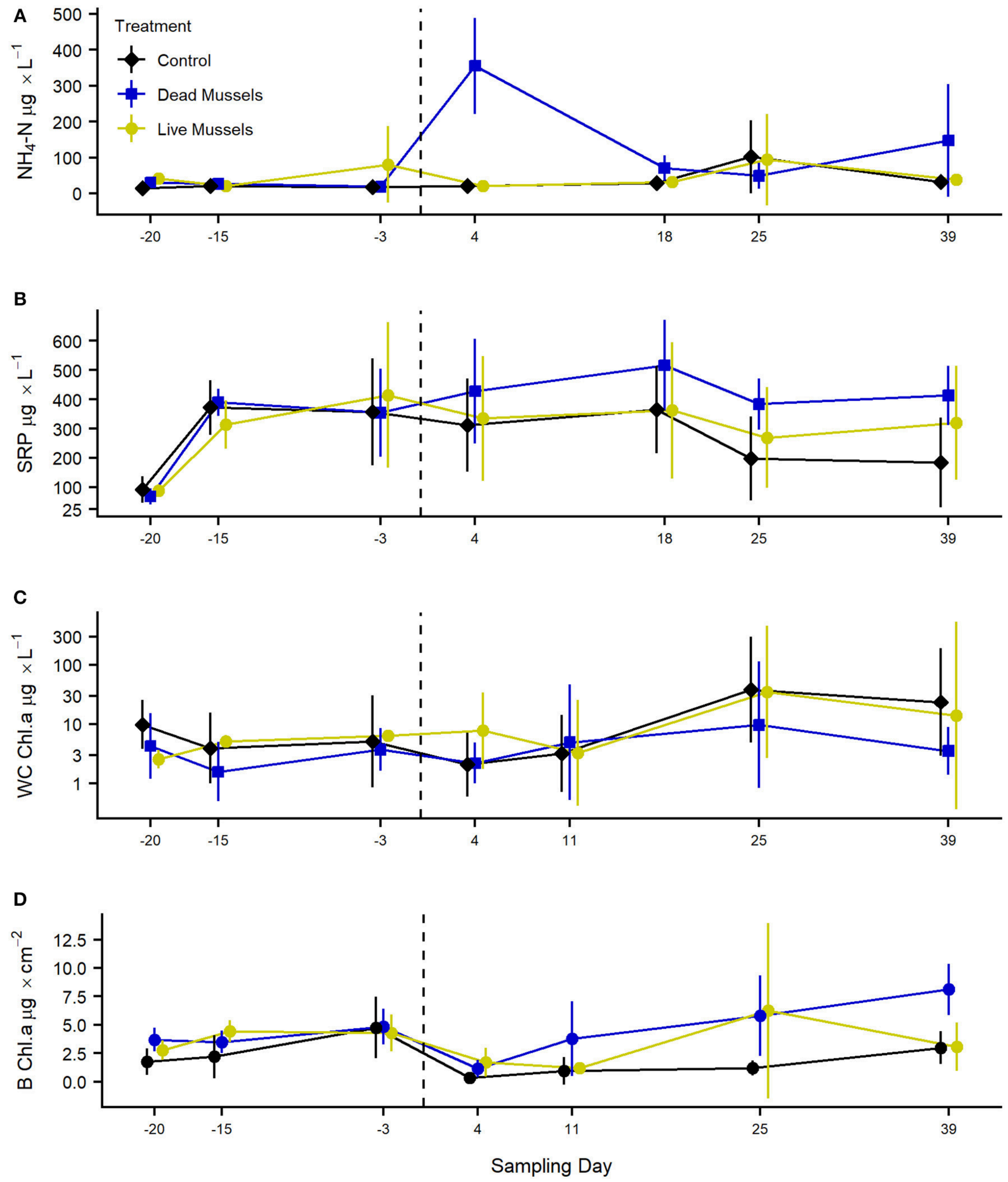


FIGURE 3 | Ambient concentrations of NH_4-N (µg L^{-1}; **A**), SRP (µg L^{-1}; **B**), water column chlorophyll *a* concentrations (µg L^{-1}; **C**) and benthic chlorophyll *a* concentrations (µg cm^{-2}; **D**) in control, live mussel, and dead mussel treatments. The dashed line represents when the mass mortality event was induced in 5 live mussel mesocosms. Points represent the mean, while the lines indicate the standard deviation. Ammonium is statistically different within control and dead treatments based on a Type III ANOVA, Tukey HSD, $p < 0.05$. Benthic chlorophyll *a* was higher in live and mortality treatments than control treatments based on a Type III ANOVA, Tukey HSD, $p < 0.05$.

areal basis (g m^{-2}). Based on our extrapolation, this mussel die-off resulted in a large pulse of both nitrogen and phosphorus (**Figure 5**). This nutrient pulse is equivalent to the areal nitrogen excretion of a mussel assemblage for 20 h, phosphorus excretion for 195 h (Atkinson et al., 2018), and to the phosphorus release from dissolving shells for two years (Wenger et al., 2018). This represents a large pulse of phosphorus that likely stimulates primary production.

Freshwater mussel soft tissue represents short term storage of nutrients since the soft tissue decays quickly, while the shell

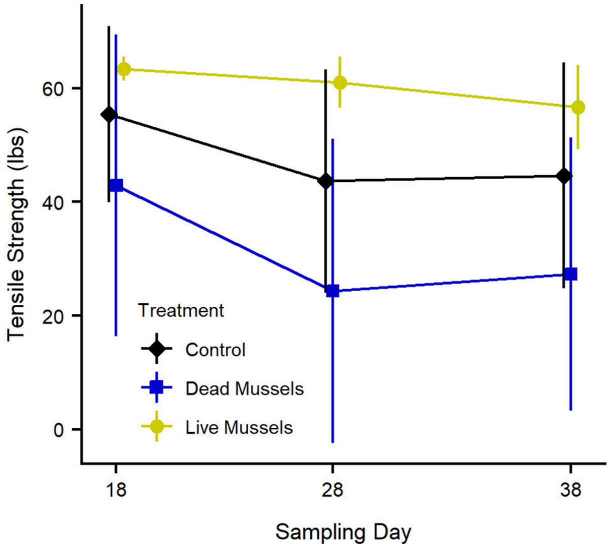


FIGURE 4 | Tensile strength (lbs.) as a proxy for decomposition of organic matter within different treatments. Cotton strips have a starting mean tensile strength of 65.61 lbs (*SD* = 1.97 lbs). Points represent the mean, while the lines indicate the standard deviation. Decomposition was higher in the mortality treatments than the live treatments and is statistically significant based on Type III ANOVA, Tukey HSD, $p < 0.02$.

represents long term nutrient storage. We wanted to determine the role of shell material as a potential long-term nutrient sink and site of nutrient release following mortality. While shell decay can be highly variable in freshwater ecosystems (Strayer and Malcom, 2007), we used the average shell decay rates from a previously published study on the Kiamichi and Little Rivers in Oklahoma (Atkinson et al., 2018) and the Sipsey River, Alabama (Atkinson, *unpublished*) to estimate spent shell biomass and nutrient release (C, N, and P) over time. Specifically, using the average decay rate ($k = -0.053$ year^{-1}) and the average shell size per site, we estimated shell biomass (g m^{-2}) over an 80-year timeframe and the subsequent nutrient release, assuming a constant rate, from spent shells following the punctuated mortality event in the Kiamichi River in 2011. We expect this estimation to be realistic as the shell decay rate was measured over a year with shell from the Kiamichi River, thus accounting for how discharge, water chemistry, and season affects shell decay rates (Strayer and Malcom, 2007; Ilarri et al., 2019).

At K3, the mass of relic shells (2.4 kg m^{-2}) exceeded shell mass of living mussels (1.6 kg m^{-2}), while all mussels in the dry reach perished, resulting in 1.7 kg m^{-2} of shell material exposed. While some shell material may have been exported due to terrestrial scavengers, we assumed that it remained in the stream channel and was submerged once flow resumed. Site K2 did not experience complete drying and the low flows did not result in as much mortality as K3 and resulted in 1.5 kg m^{-2} of relic shell while 5.0 kg m^{-2} was maintained in live mussels. Based on the modeling described above, shell material decay (**Figure 6A**) would result in a slow nutrient release at each of these sites (**Figures 6B–D**). For example, 413 g C m^{-2}, 4.8 g N m^{-2}, and 0.4 g P m^{-2} remained in the shell of dead mussels at K3 in the reach that did not dry, which would then be slowly released by shell decayed (**Figures 6B–D**). The surviving, living mussels at K3 still maintained nutrients in their shell and continued to store nutrients and potentially grow and store additional material. When mortality is equal to the production of shells, shell mass is maintained at a steady state. But large-scale die-offs lead to a pulse in relic shells and lower production of shell material. This represents a long-term loss of mussel-driven nutrient storage and shell habitat within stream reaches (Wenger et al., 2018).

IMPLICATIONS AND CONCLUSIONS

Based on the presented case studies, our mesocosm experiment, and our extrapolated nutrient pulse due to a mussel die-off, we conclude that stream ecosystems are severely altered following mussel mass mortality events. Mussel loss is governed by drought severity, location within the river network, and species-specific drought tolerances. In the short term, decomposing carrion from mussel die-offs releases a large pulse of nutrients into the water which stimulates food web productivity. In the long term, the overall loss of mussel biomass, and the loss of functional traits as more sensitive species decline, leads to decreases in ecosystem function which may take decades to recover (**Figure 1**).

While we have frequently observed algal blooms in the field following mussel die-offs, we did not observe algal blooms within our mesocosm experiment. In our small mesocosms, the decomposition of mussel tissue likely altered the microbial community to favor heterotrophs, which potentially outcompeted water column algae for available nutrients. Our extrapolation from the observed mussel die-off in the Kiamichi River predicted a large phosphorus pulse. After inducing a die-off of the invasive bivalve *Corbicula*, McDowell et al. (2017) observed a smaller increase in ambient phosphorus concentration than expected. They posited that algal uptake of SRP accounted for difference between the predicted increase in water column SRP and what was measured. Further exploration of the interacting factors driving algal bloom formation after mussel die-offs during drought is warranted.

The frequency and severity of hydrologic drought is predicted to increase in the southcentral and southeastern U.S. as a consequence of climate change and increasing human demand for water (Baron et al., 2002; Golladay et al., 2016). This region also contains the highest diversity of freshwater mussels globally (Williams et al., 1993; Haag, 2010). Thus, future mussel mass mortality events are highly likely and we need to both understand their ecological effects and how to mitigate them. Individual mussel species' tolerances to maximum water temperature and minimum dissolved oxygen concentrations vary and are an area of active research (Archambault et al., 2014; Jeffrey et al., 2018). Understanding how mussels acclimate and potentially adapt to increased water temperatures and reduced water availability will be critical to protecting this diverse guild (Galbraith et al., 2012; Gough et al., 2012). However, it is unlikely that the drought sensitive species will rebound to their former abundance without

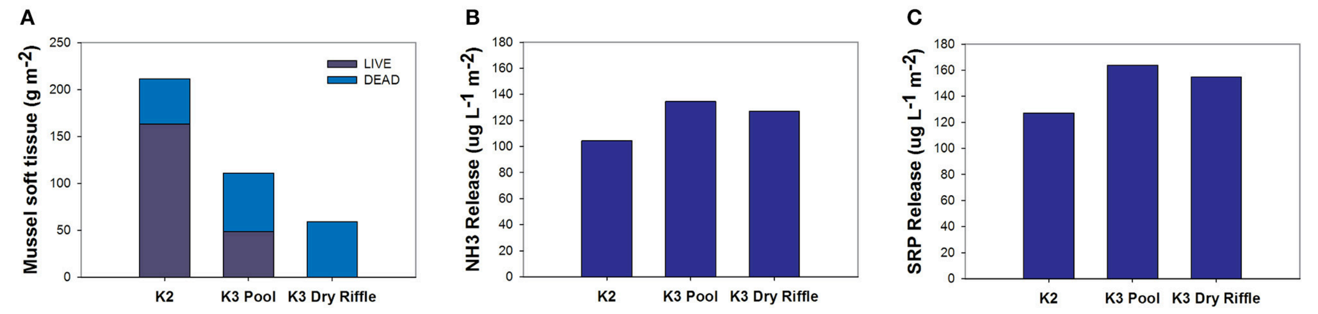


FIGURE 5 | Extrapolated nutrient release from Kiamichi mussel beds after a drought-induced mass mortality event. The shell lengths from dead unionids are used to predict mussel soft tissue **(A)**, which was then paired with nutrient data from the mesocosm experiment to predict ammonium release (μg m^{-2}; **B**) and SRP release (μg m^{-2}; **C**).

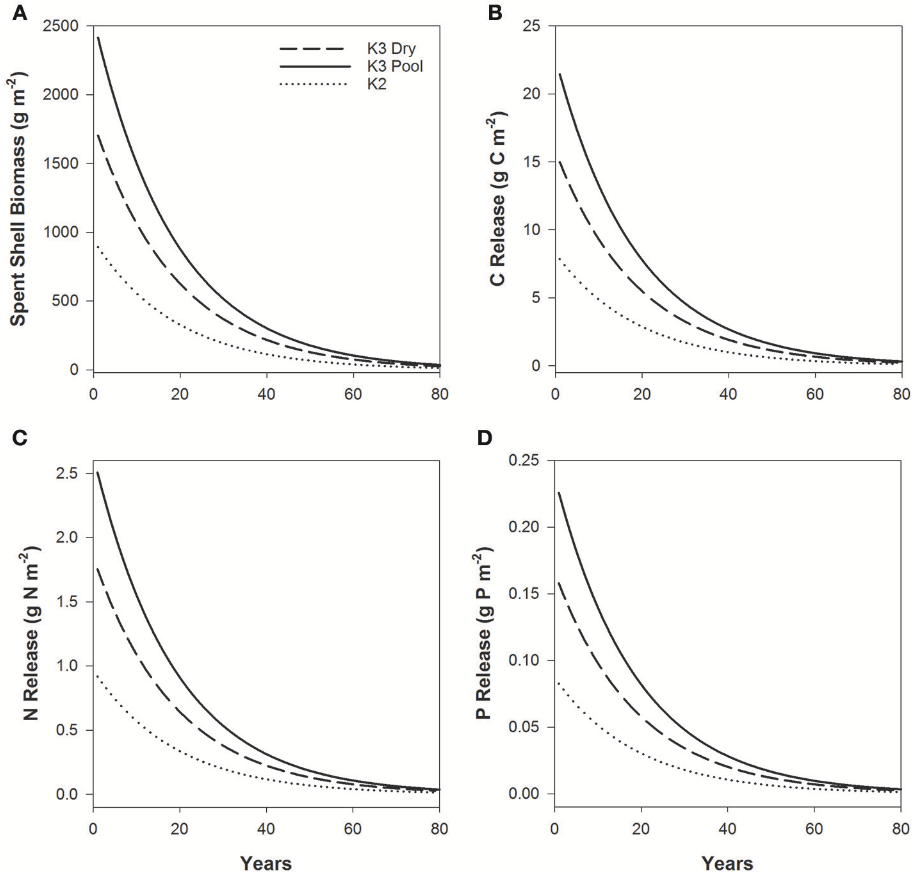


FIGURE 6 | Shell decomposition **(A)** of spent mussel shells following the mass mortality event as a result of low flow and high temperature conditions at 3 sites in the Kiamichi River, OK and the resultant release of carbon **(B)**, nitrogen **(C)**, and phosphorus **(D)** from the shell material over time. Shell decomposition is modeled based on the shell decomposition rates and shell stoichiometry values from Atkinson et al. (2018) and the empty shells sampled from Southeastern Oklahoma in the drought of 2011.

changes in water management and restoration of populations through mussel propagation.

Freshwater mussels are not the only organism threatened by mass mortality events and rivers are not the only ecosystem altered through these events. Our research provides an example of how the loss of an abundant, long-lived organism has cascading and long-term impacts on ecosystems. These impacts are analogous to loss of a forest in terrestrial ecosystems; habitat provision and nutrient sequestration is altered as the community shifts and takes decades to rebound (Ellison et al., 2005; Boyd et al., 2013). The loss of this long-lived organism and the subsequent release of this nutrient pulse has large impacts on stream ecosystems.

ETHICS STATEMENT

Fish were handled in accordance with the recommendations of American Fisheries Society's Guidelines for the use of Fishes in Research. The protocol was approved by the University of Oklahoma's Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

TD, CV, and CA designed the mesocosm experiment and TD performed it. CA, CV, and SG provided field data on mussel losses and shell dissolution rates. TD performed analyses and wrote the manuscript with input from all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00274/full#supplementary-material

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REVIEW
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Mass Mortality Events of Invasive Freshwater Bivalves: Current Understanding and Potential Directions for Future Research

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Mass mortality events, the rapid, catastrophic die-off of organisms, have recently been recognized as important events in controlling population size, but are difficult to quantify given their infrequency. These events can lead to large inputs of animal carcasses into aquatic ecosystems, which can have ecosystem scale impacts. Invasive freshwater bivalves such as the Asian clam *Corbicula fluminea*, the zebra mussel *Dreissena polymorpha*, the golden mussel *Limnoperna fortunei*, and the Chinese pond mussel *Sinanodonta woodiana* can attain high densities and biomass and play important roles in aquatic ecosystems through filtration, bioturbation, and excretion. Invasive bivalve species can best be described as R-selected species and appear not to have the same tolerance to abiotic stressors as native species, causing them to be prone to mass mortality events in their invasive range. In contrast to their ecological effects while alive, the frequency and impacts of mass mortality events of invasive freshwater bivalves are not well-understood. Here we review the causes and impacts of mass mortality events, as well as identify important questions for future research. Extreme abiotic conditions, including both drought and flooding, as well as high and low temperatures were the primary drivers of mass mortality events. Short-term impacts of mass mortality events include large pulses of nitrogen and increased oxygen stress due to large amounts of soft tissue decomposition, while shells can impact habitat availability and nutrient cycling for decades. Impacts on biological communities (bacteria, fungi, and macroinvertebrates) are less studied but some examples exist concerning *C. fluminea*. Better documentation of mass mortality events, particularly their magnitude and frequency, is needed to fully understand the impacts invasive bivalve species have on ecosystems, especially as climate change may make mass mortality events more frequent and/or have a larger magnitude.

Keywords: boom-bust dynamics, die-off, extreme events, invasive species, non-indigenous species, *Corbicula*

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BACKGROUND

Mass mortality events, "the rapid, catastrophic die-off of organisms that punctuate background mortality rates" (Fey et al., 2015), have recently been recognized as important events in controlling population size, but it can be difficult to put the significance of these events in a broader context given their infrequency (Fey et al., 2015). A meta-analysis showed that mass mortality events are increasing in both frequency and magnitude across several taxa, including marine invertebrates (Fey et al., 2015); however, freshwater invertebrates were not included within this study. For invasive species, research often focuses on quantifying effects as a function of their range, abundance, and per capita impact (Parker et al., 1999). In doing this, much research has focused on their impacts while alive, without capturing the impacts of possible periodic mortality events.

Within freshwater ecosystems, bivalves such as clams and mussels play a critical role in a wide range of ecosystem functions, including filtering bacteria, particulates, and primary producers from the water column, bioturbation via movement and pedal feeding, and excretion of important nutrients such as nitrogen and phosphorus (Covich et al., 1999; Vaughn and Hakenkamp, 2001; Vaughn and Hoellein, 2018). Many of these processes directly benefit humans as ecosystem services, such as removing nutrients and sediment from water (Vaughn, 2018). Globally, freshwater mussels (Bivalvia, Unionida) are among the most threatened organisms on earth (Strayer et al., 2004; Lopes-Lima et al., 2017, 2018) and many populations are experiencing large scale declines (Haag, 2012; Ferreira-Rodríguez et al., 2019). In many ecosystems, declines of native mussels have been coupled with the establishment of invasive bivalve species that evolved elsewhere in the world and now have negative economic and ecological impacts (Strayer and Malcom, 2018).

Invasive bivalves, including the zebra mussel *Dreissena polymorpha*, the golden mussel *Limnoperna fortunei*, the Asian clam *Corbicula fluminea*, and the Chinese pond mussel *Sinanodonta woodiana*, have been shown to have dramatic ecological and economic impacts on freshwater ecosystems and are among the most widespread and damaging invasive species in the world (Sousa et al., 2014). Many invasive bivalve species have large-scale impacts on ecosystem function (Caraco et al., 1997; Strayer et al., 1999; Zhu et al., 2006) and can act as ecosystem engineers (Sousa et al., 2009). They can also often times reach extremely high densities of several hundreds to thousands of individuals per square meter (Caraco et al., 1997 for *D. polymorpha*, McDowell and Byers, 2019 for *Corbicula* sp., Bódis et al., 2016 for *Sinanodonta woodiana*), and sometimes even on the order to hundreds of thousands of individuals per square meter for the golden mussel *Limnoperna fortunei* (Sylvester et al., 2007). Invasive bivalves have also been observed to die en masse (Ilarri et al., 2011), with sometimes 90–99% of individuals dying within a short period of time (Haag and Warren, 2008; McDowell et al., 2017). This leads to millions of individuals dying at once, with their bodies releasing nutrients within a few days (McDowell et al., 2017).

Our understanding of the importance and impacts of animal carcasses in aquatic ecosystems remains incomplete. Pulses of nutrients into systems can have major bottom-up impacts on food webs (Yang, 2004), especially in aquatic systems where nutrients can move rapidly through the food web (Nowlin et al., 2008). Although a great deal of research has focused on the importance of carcasses of anadromous salmon to aquatic ecosystems and adjacent forests (e.g., Helfield and Naiman, 2002; Hocking and Reynolds, 2011) in the Pacific Northwest region of the United States, examples on other faunal groups and continents are less frequent (but see Subalusky et al., 2017). However, recent research has highlighted the importance of the remains of animals in freshwater ecosystems, and the impacts of the reduction of animal remains in freshwater ecosystems due to overall population declines (Wenger et al., 2019). For example, Wenger et al. (2019) estimate that dissolution of mussel shells could have provided 1% of the total phosphorus load in rivers during median flow and typical shell production rates; periodic mass mortality events that generate more shell might have played a more important role. Even less studied are the possible impacts of massive mortalities by invasive species in the invaded range.

From an ecological theory perspective, invasive bivalves may be prone to frequent, episodic mortality events due to their tendency to be “weedy” R selected species that have lower tolerance to abiotic stressors (McMahon, 2002), which may make them more prone to “boom-bust” dynamics where populations undergo large fluctuations (Strayer et al., 2017). The “boom-bust” model of invasive species has been documented in a variety of organisms, including plants (Stott et al., 2010), insects (Lester and Gruber, 2016), and molluscs (Moore et al., 2012).

In order to better understand the roles of mass mortality events of invasive bivalves in aquatic ecosystems, here we: 1. Review the known mass mortality events of invasive freshwater bivalves, including their causes, impacts, and whether or not native species were affected; and 2. Identify nine important questions for future research on mass mortality events of invasive freshwater bivalves.

CAUSES OF MASS MORTALITY EVENTS

We identified documented mass mortality events of invasive bivalves through a literature review searching for studies examining “mass mortality,” “die-offs,” or “population declines” of invasive bivalves in freshwater ecosystems using Google Scholar over any time period. In particular, we focused on the following invasive bivalves that are abundant and widespread: the Asian clam *Corbicula* sp., the zebra mussel *Dreissena polymorpha*, the golden mussel *Limnoperna fortunei*, and the Chinese pond mussel *Sinanodonta woodiana*. All studies identified may be found in **Table 1**. It is important to note that mass mortality events are not unique to invasive bivalves, and can also occur with native bivalve species (e.g., Vaughn et al., 2015 in Oklahoma, Sousa et al., 2018 in the Iberian Peninsula). While this review focuses on mass mortality events of invasive bivalves, a critical and open research question is to what extent the responses of native and invasive bivalves may differ and or interact (DuBose et al., 2019). Generally speaking, mass mortality events of invasive bivalve species were triggered by extreme abiotic conditions, including drought, flood, extreme high temperatures, and extreme low temperatures (**Figure 1**).

Drought and High Temperatures

Drought and high temperatures, which typically co-occurred and therefore cannot be split apart as separate stressors, were the dominant driver of mass mortality events of invasive freshwater bivalves. We found documented high temperature mortality events in the southern United States in particular

TABLE 1 | Summary of documented mass mortality events of invasive bivalves. Studies are sorted by cause, then species.

References	Location	Habitat	Species	Cause
Golladay et al. (2004)	GA, USA	Stream/river	*Corbicula*	Drought/heat
Gagnon et al. (2004)	GA, USA	Stream/river	*Corbicula*	Drought/heat
Haag and Warren (2008)	AL and MI, USA	Stream/river	*Corbicula*	Drought/heat
Atkinson et al. (2014)	OK, USA	Stream/river	*Corbicula*	Drought/heat
McDowell et al. (2017)	GA, USA	Stream/river	*Corbicula*	Drought/heat
Mouthon and Daufresne (2006)	France	Stream/river	*Corbicula*	Drought/heat
Foekema et al. (2008)	The Netherlands	Stream/river	*Corbicula*	Drought/heat
Ilarri et al. (2011)	Portugal	Stream/river	*Corbicula*	Drought/heat
Bódis et al. (2014a)	Hungary	Stream/river	*Corbicula, S. woodiana*	Drought/heat
Balogh et al. (2008)	Hungary	Lake/Reservoir	Zebra mussel	Drought/heat
Churchill (2013)	TX and OK, USA	Lake/Reservoir	Zebra mussel	Drought/heat
Churchill et al. (2017)	TX and OK, USA	Lake/Reservoir	Zebra mussel	Drought/heat
White et al. (2015)	MI, USA	Lake/Reservoir	Zebra mussel	Drought/heat
Sousa et al. (2012)	Portugal	Stream/river	*Corbicula*	Flood
Castañeda et al. (2018)	Canada	Stream/river	*Corbicula*	Low temperatures
Smith et al. (2018)	WI, USA	Stream/river	*Corbicula*	Low temperatures
Werner and Rothhaupt (2008)	Switzerland	Lake/Reservoir	*Corbicula*	Low temperatures/desiccation
Leuven et al. (2014)	The Netherlands	Lake/Reservoir	*Corbicula*, Zebra mussel	Low temperatures/desiccation

(Georgia: Gagnon et al., 2004; Golladay et al., 2004; McDowell et al., 2017, **Figure 2A**; Alabama and Mississippi: Haag and Warren, 2008; Oklahoma: Atkinson et al., 2014; Vaughn et al., 2015; Texas and Oklahoma: Churchill, 2013; Churchill et al., 2017). High summer temperatures were also associated with mortality events for *C. fluminea* in both Portugal (Ilarri et al., 2011) and France (Mouthon and Daufresne, 2006), as well as a mortality event in Hungary which affected *Corbicula* sp., *S. woodiana*, and native mussels (Bódis et al., 2014a, **Figure 2B**). During drought events, small streams may be impacted more heavily, as they are more prone to dewatering; following a drought in Alabama and Mississippi, *C. fluminea* populations declined 90–98% in small streams, but significantly increased in two of the three large river sites studied (Haag and Warren, 2008).

The exact trigger for the mortality can vary from case to case. High temperatures and drought can lead to bivalves being stranded on sand bars or within disconnected pools as flows decline (Atkinson et al., 2014), or even mortality of individuals still within flowing water, due to high temperatures and low dissolved oxygen concentrations (Foekema et al., 2008 as cited in Ilarri et al., 2011; Leuven et al., 2014; McDowell et al., 2017). In estuarine areas, drought can lead to increases in salinity, which may be a contributing factor to *Corbicula* mass mortality events within the Minho River in Portugal (Ilarri et al., 2011). Within lakes, drought can lead to water level fluctuations, leaving individuals stranded, as was observed in a zebra mussel mortality event in Lake Balaton, Hungary (Balogh et al., 2008) and in Lake Texoma on the border between Texas and Oklahoma, USA (Churchill, 2013; Churchill et al., 2017). Mortality events may occur regularly during heat waves and with a large enough magnitude to control population sizes, as Ilarri et al. (2011) noted that the lowest observed population densities of *C. fluminea* corresponded to years with summer heat waves. Mortality events can also occur at temperatures that are "sub-lethal" per laboratory experiments (White et al., 2015), emphasizing the importance of field research on these events.

Flooding

Flooding, the other hydrologic extreme, can also lead to mass mortality events. Floods typically do not directly kill the bivalves, but instead transport them to unsuitable habitats during high flows after which they are stranded when flood waters recede. High flow events can play an important role in controlling the distribution of a bivalve within a river (Strayer, 1999), and can lead to mass mortality events of invasive species (Sousa et al., 2012, **Figure 3**). Flooding during the winter months in northern Portugal led to deposition of up to 2,200 individuals m^{-2} and 10,200 g^{-2} wet biomass on adjacent river banks. *Corbicula fluminea* was the most common species transported during flood mortality events, both by biomass and density, despite *C. fluminea* not being the dominant bivalve at some of the study sites (Sousa et al., 2012).

Low Temperatures

Extreme cold can also lead to mass mortality events of freshwater bivalves. Though minimum temperatures have been shown to be an important controller for the distribution of *C. fluminea* through modeling (McDowell et al., 2014) mass mortality events induced by low temperature are often associated with human manipulations of temperature or water level. In Europe, an extremely cold winter coupled with low water levels in Lake Constance led to a 99% mortality rate for *Corbicula* that were either stranded or in water up to 3 m deep (Werner and Rothhaupt, 2008). Similarly, low water levels and cold temperatures led to high mortality of zebra mussels within

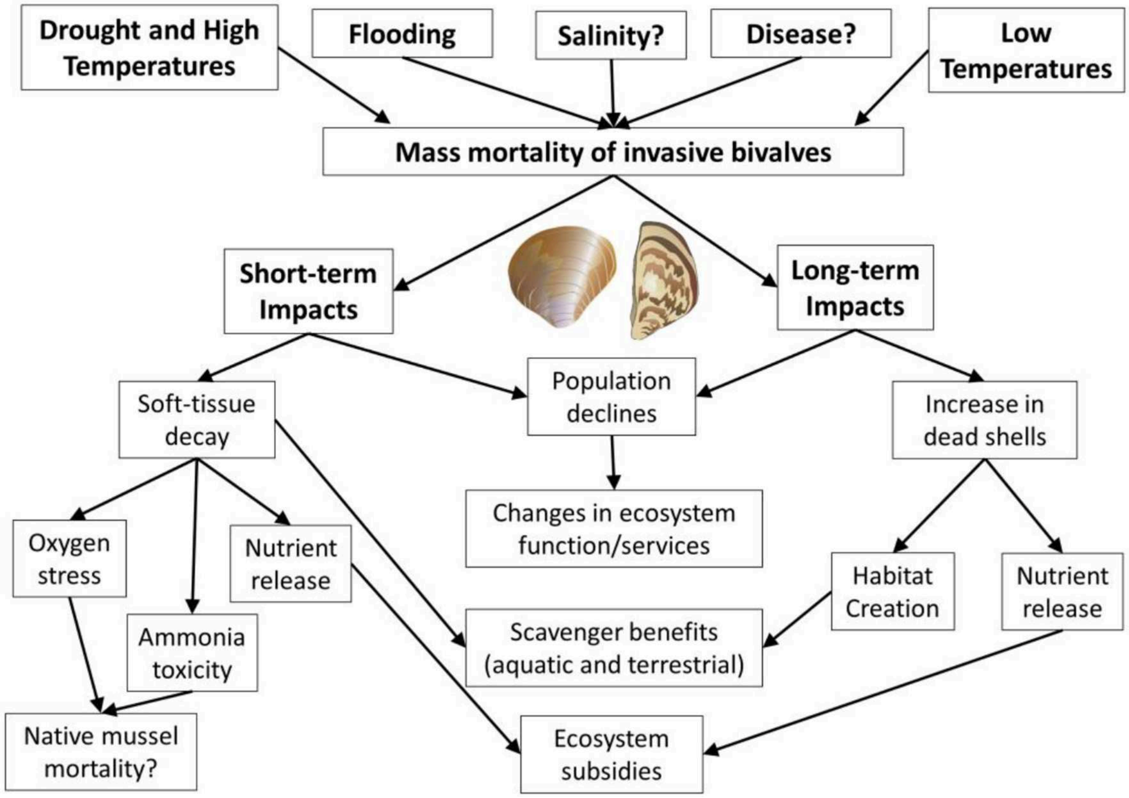

FIGURE 1 | Conceptual diagram of the causes of mass mortality events of invasive bivalves, as well as their short- and long-term impacts. Disease and salinity are included as potential causes of mass mortality events, but have not been documented. Repeated mass morality events could drive down population sizes, particularly if their magnitude or frequency increases due to climate change, though mass mortality events driving long term population declines is hypothetical. Images of Corbicula and zebra mussels are courtesy of the IAN Symbol Libraries (http://ian.umces.edu/symbols/).

impounded sections of the Rhine and Meuse Rivers in the Netherlands (Leuven et al., 2014). The combination of water level drawdowns and low temperatures has been used as a mechanism to control zebra mussel populations (Grazio and Montz, 2002, as cited in Leuven et al., 2014). Conversely, while industrial cooling water plumes may provide thermal refuge for invasive species in areas that are otherwise climatically unsuitable, the cessation of industrial activities can lead to rapid extirpation of populations that relied on the artificial warming to persist. Both individuals and populations can thrive in thermal effluent—one study in the River Shannon, Ireland found that within thermal plumes individuals were more than twice as large by length and body mass, densities were more than 13 times higher, and biomass was nearly 50 times higher (Penk and Williams, 2019). In North America, a population of *Corbicula* was well-established within the St. Lawrence River in the cooling water plume of a power plant (Simard et al., 2012), but a rapid extirpation followed the decommissioning of the power plant (Castañeda et al., 2018). Mortality events can also occur despite thermal refuge during particularly severe winters, as was documented in the Great Lakes region of the United States with a near extirpation of *Corbicula* in the Fox River (Smith et al., 2018).

IMPACTS OF MASS MORTALITY EVENTS

The most immediate response to mass mortality events is the input and subsequent decomposition of dead tissue into ecosystems (**Figure 1**). The largest bivalve mortality event documented was caused by drought conditions in the Danube River and created an input of over 20–30 kg m^{-2} of wet biomass (which includes shells) and over 1,000 g m^{-2} of ash free dry mass at the sites with the highest densities of invasive bivalves (Bódis et al., 2014a). This mortality event primarily affected the Chinese pond mussel *S. woodiana*, but also included two other invasive bivalves: *Corbicula* sp. and the zebra mussel *D. polymorpha*. The decay of soft tissues can be quite rapid, on the order of days (McDowell et al., 2017), and this rapid decay, particularly during warm summer months, could exacerbate already stressful oxygen conditions (Gagnon et al., 2004; McDowell et al., 2017).

Short-Term Effects

During the decay of soft tissues decay, large amounts of nutrients are released, creating a pulse of resources (Sousa et al., 2012). This can lead to large scale shifts on both a local and ecosystem level. McDowell et al. (2017) estimated that a mortality event of ~100 million *Corbicula* released ~751 kg of carbon, 180 kg of nitrogen,

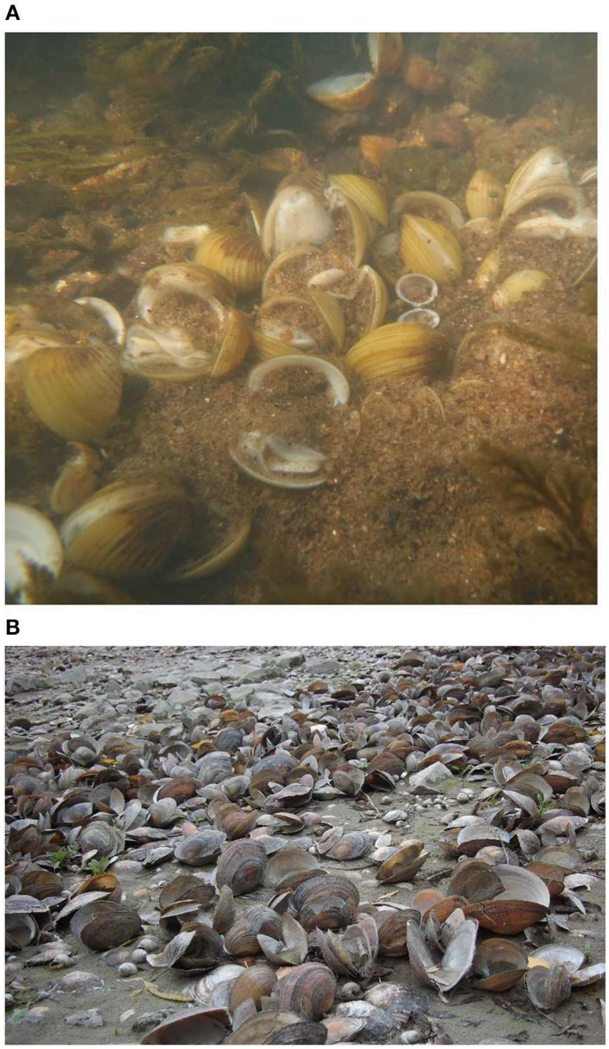

FIGURE 2 | (A) *Corbicula* die-off in Georgia USA caused by a summer drought, described in McDowell et al. (2017). Note the presence of soft tissue. **(B)** *Sinanodonta woodiana* die-off in the Danube River (Hungary) after a drought in 2011. Described in Bódis et al. (2014a).

FIGURE 3 | Accumulation of *C. fluminea* after the 2001 flood in the banks of Minho River. This photo was taken in 2014, 13 years after the flooding, showing the longer-term impacts that additions of shells can have on an ecosystem.

and 45 kg of phosphorus as soft tissues decayed. The release of nutrients from a mass mortality event can lead to potentially toxic concentrations of unionized ammonia for unionid mussels, in both the water column (Cherry et al., 2005) and the porewater of sediment (Cooper et al., 2005). However, in a manipulative study simulating a die-off of *C. fluminea* in Minho River (Iberian Peninsula) no changes were detected in the structure of aquatic microbial and invertebrate communities nor litter breakdown rate (Novais et al., 2017).

Long-Term Effects

The impacts of the decay of soft tissue can be quite large, but short lived, whereas the impacts of shells could extend for decades, given their slow breakdown rate (Strayer and Malcom, 2007; Ilarri et al., 2015). The time frame for the impact of additional shells can vary substantially depending on whether or not they are found in a terrestrial or aquatic ecosystem, as the decay rate for a variety of bivalve shells (including *C. fluminea*) is six to twelve times faster in aquatic systems than in terrestrial (Ilarri et al., 2015). Shells, and the trickled release of nutrients, could play an important role in the biogeochemistry of rivers and adjacent flood plains, as bivalve shells can provide significant storage of nutrients, particularly carbon and nitrogen (Vaughn et al., 2015). While shells are relatively poor in both nitrogen and phosphorus [$\sim$1% N and 0.01% P for unionid mussels Christian et al., 2008; Atkinson et al., 2010] compared to soft tissue, the sheer mass of shells can provide a significant release of nutrients over time. Wenger et al. (2019) estimated that based on historical densities, the breakdown of mussel shells once provided $\sim$1% of the total phosphorus load in rivers in the southeastern United States.

Habitat Creation

The shells of invasive freshwater bivalves can alter the physical habitat within a river, contributing to their role as ecosystem engineers (Sousa et al., 2009). Within the Danube River, empty bivalve shells increased the abundance of a variety of macroinvertebrates. For rivers that are dominated by fine sediment, these shells can provide important habitat for benthic invertebrates and increase their abundance and biomass (Bódis et al., 2014b). Other studies have shown that shell density of *C. fluminea* is positively associated with biomass, diversity, and density of macroinvertebrates (Ilarri et al., 2012). Shells resulting from die-offs can also have significant effects on estuarine and freshwater macroinvertebrates than can use them as substrate or as refuge from predators (e.g., Ilarri et al., 2012, 2014, 2018; Bódis et al., 2014b; Novais et al., 2015).

Foodweb Alterations

Given the importance of filter feeding bivalves in aquatic foodwebs (Vaughn and Hakenkamp, 2001; Vaughn and Hoellein, 2018), it is unsurprising that mass mortality events would alter foodwebs. We have identified two main ways these foodweb alterations can occur: direct benefits to scavenging organisms and ecosystem subsidies from aquatic to terrestrial ecosystems.

Scavenger Benefits

The addition of soft tissue should be a benefit for scavenging species as well, though published reports of this are limited. Mouthon (2001) reported that soft tissue from *Corbicula* mortality events was consumed by silurid fishes, though to our knowledge that is the only published account documenting this behavior. Anecdotally, researchers and fishermen have reported estuarine fish moving upstream in Portuguese rivers to consume *C. fluminea* corpses (Sousa personal observation) and within Georgia rivers, flocks of crows have been observed consuming dead *Corbicula* that were stranded on a sandbar (McDowell personal observation). Any aquatic scavengers such as crayfish ought to benefit as well, but again, this has not been reported in the literature and remains an open question (see below).

Ecosystem Subsidies

Nutrients from bivalve decay often cross ecosystem boundaries, providing important subsidies that span the terrestrial aquatic interface. Subsidies from aquatic to terrestrial systems play an important role in many ecosystems, often through anadromous fish such as salmon (Helfield and Naiman, 2002). Mass mortality of invasive freshwater bivalves, particularly through drought or flooding, can lead to ecosystem subsidies of energy and nutrients to terrestrial ecosystems (Sousa et al., 2012; Bódis et al., 2014a, **Figure 3**). Addition of carrion can impact both nutrient dynamics and communities, as two studies (Novais et al., 2015, 2017) simulating massive die-offs of *Corbicula* in terrestrial ecosystems after floods showed clear effects on soil chemistry, fungal biomass, and bacterial, fungal, and macroinvertebrate communities. Pulses of nutrients could also increase insect emergence rates in response to additions of phosphorus (Mundie et al., 1991) and both nitrogen and phosphorus (Blumenshine et al., 1997) in experimental stream mesocosms. These higher emergence rates could be an additional way that mass mortality events strengthen subsidies to terrestrial ecosystems, as these insects provide an important food source for riparian predators (Baxter et al., 2005).

Alterations to Ecosystem Function

Given the importance of filter feeding bivalves in aquatic ecosystems (Vaughn and Hakenkamp, 2001; Vaughn, 2018), including filtration, bioturbation, and nutrient storage, a mortality event can also dramatically alter ecosystem function. For example, within a Georgia River, overall filtration rates by *Corbicula* dropped 99.6% following a mass mortality event (McDowell et al., 2017). Filtration is a particularly important function of freshwater bivalves, as it removes particles from the water column and connects the water column and benthic food webs (Vaughn and Hakenkamp, 2001). Few studies have focused on shifts in ecosystem function following mortality of invasive freshwater bivalves, but Vaughn et al. (2015) showed that multiple severe droughts over 20 years caused native mussel mortality that substantially reduced the ecosystem function they provided. Losses were disproportionately felt by thermally sensitive species, which had higher percentages of both nitrogen and phosphorus in their tissues (Atkinson et al., 2014). These impacts are driven by the massive decline in population size, and therefore the overall role of the species in the ecosystem, and can be short-term and transient if the populations recover, or long-term if the population size remains low or is unable to fully recover due to repeated mortality events. It is unclear if these changes at the ecosystem level will return systems to function closer to their pre-invasion status thanks to the diminished role of invasive species, or if the dramatic decline in filter feeding bivalves will push these systems farther from historical function, even though the dominant bivalves are invasive. Although we are not aware of quantitative studies, similar effects are possible in response to massive declines in density and biomass of dominant invasive freshwater bivalves after massive die-offs.

OPEN QUESTIONS

In attempting to understand the overall causes and impacts of mass mortality events of invasive freshwater bivalves, we identified several important open questions which researchers should/may address in future studies.

How Frequent Are Mass Mortality Events of Invasive Bivalves?

To date, most research has focused on quantifying the magnitude and impacts of mass mortality events, rather than their frequency. In addition to the impacts described above, if ecosystems are experiencing regular mortality events, this could also lead to depressed population sizes, and therefore reduce the impacts of invasive species on ecosystem function. Ilarri et al. (2011) found some indication that mass mortality events temporarily depressed population sizes, as *C. fluminea* densities were the lowest in years following notable heat waves. Overall, it is not clear if mass mortality events are unusual, and best described as a "solitary boom-bust" model in which the population of an invasive expands rapidly, only to decline (Strayer et al., 2017), or if mass mortality events are regular occurrences, best fitting either the "recurrent" or "cyclic" models of boom-bust dynamics where population declines are occurring at least twice, and may occur at regular intervals (Strayer et al., 2017).

The responses of the invasive bivalves to mortality events may vary due to different life spans, with *S. woodiana* having a much longer life span [∼12 years Dudgeon and Morton, 1983], than *Limnoperna fortunei* [2–3 years Ricciardi, 1998] or *Corbicula* and zebra mussels [∼3–5 years McMahon and Bogan, 2001; Strayer and Malcom, 2006], so it would be expected that the recovery trajectories differ as well. For several of these species, we do not have a long enough period of monitoring to determine long term responses to mass mortality events. In addition to possible differences in responses and recovery from mortality events at the species level, populations from different latitudes or subjected to different abiotic conditions (e.g., food resources, altitude; Crespo et al., 2015) may respond differently following a mortality event. Assuming that invasive populations are able to recover, the frequency of mass mortality events fundamentally alters the balance of the impacts invasive bivalves have while alive (filtration, storage and excretion of nutrients, bioturbation) and the impacts they have during mass mortality events (nutrient release, availability of empty shells, loss of filtration). A critical component to answering this question will be better monitoring of mass mortality events; for *Corbicula* sp., for example, anecdotal evidence indicates that mortality events are relatively common, although they are not well-described in the literature (McDowell et al., 2017). In some cases, mortality of invasive species is only briefly described as part of a study more heavily focused on native species (e.g., Vaughn et al., 2015). In order to better assess the impact and novelty of mass mortality events as part of our overall understanding of the impact of invasive species, better quantitative documentation is needed, and in particular we must know:

Are Native Species Affected Similarly to Invasive Bivalves?

Invasive species are often considered "weedy," with high reproduction rates but poorer tolerance to stressful abiotic conditions than native species, so therefore ought to experience more frequent mortality events (McMahon, 2002). It is important to note, however, that native species do not represent a homogenous group. Within the United States, the native unionid mussels vary substantially in their ability to tolerate a wide variety of stressors such as desiccation, high temperatures, or low dissolved oxygen (Haag, 2012). Differences in abiotic tolerances should lead to differences in the frequency and magnitude of mortality events in communities dominated by invasive species compared to those with an intact mussel community, especially as an intact native community ought to have a more heterogeneous response to abiotic stressors. Using a trait based framework for the bivalve community as a whole could be an illuminating way to compare the differing roles in and impacts on ecosystems that native and invasive bivalves have in the face of extreme events (de Bello et al., 2010). This approach could lead to broader, more generalizable results than explicit pairwise comparisons of native and invasive species (McGill et al., 2006). Few studies have quantified the mortality of both native and invasive bivalves, but those have generally shown a higher mortality rate for the invasive species induced by both drought (Haag and Warren, 2008; Bódis et al., 2014a) and flood (Sousa et al., 2012). In contrast to the "missing dead"—a decline in the number of animal carcasses in aquatic ecosystems due to widespread population declines—described by Wenger et al. (2019), if mass mortality events of invasive species are occurring more frequently or to a larger magnitude than we would expect in an uninvaded community, mortality events could represent a source of "found dead" instead, increasing overall inputs of shell and soft tissue into aquatic ecosystems. While baseline mortality would be contributing shell and soft tissue, repeated massive mortality followed by rapid population growth could increase the overall amount of inputs of tissue and shell into the system.

Is *Corbicula* sp. Less Tolerant to Abiotic Stressors Than Other Invasive Bivalves?

Most of the documented mass mortality events of invasive bivalves are for *Corbicula* sp., and include populations in Europe and North America. A key question that remains to be answered is if *Corbicula* is particularly vulnerable to mass mortality events or whether *Corbicula* dominates studies of mass mortality of invasive bivalves because it has been a research focus for multiple groups in Portugal, Georgia, and Oklahoma. Many of the documented mass mortality events occurred in the southern United States or in southern Europe—is the frequency of mass mortality events a product simply of the extreme heat during the summers in these regions? Mortality events for *Corbicula* were also primarily in lotic systems, so it is possible that populations in lotic systems are more vulnerable due to fluctuations in water levels and possibly food resources, which will decrease the physiological capacity to deal with a stressful event such as heatwaves. In some instances, mortality events were associated with cessation of flow, creating disconnected pools which often led to mortality of native mussels as well (Atkinson et al., 2014). This could lead to both high temperature and low oxygen, though *Corbicula* sp. is more tolerant of hypoxia than the zebra mussel at temperatures up to 25°C (Matthews and McMahon, 1999). Using laboratory experiments to better define the tolerance of *Corbicula* to stressful abiotic conditions, particularly high temperatures and low dissolved oxygen, is particularly important to help protect and conserve native bivalves, as the mortality of *Corbicula* could exacerbate the negative impacts of mass mortality events on native species via increased oxygen stress (Gagnon et al., 2004 in south Georgia) and toxic concentrations of ammonia (Cherry et al., 2005; Cooper et al., 2005).

In contrast, we did not find any documented mass mortality events of *L. fortunei* and a small number impacting *D. polymorpha*. Further research is needed in order to determine if *L. fortunei* and *D. polymorpha* follow similar patterns to *Corbicula* or *S. woodiana*, which have large-scale mortality events. The golden mussel in particular appears to be very tolerant of a wide range of abiotic conditions, including high temperatures, low dissolved oxygen, and acidic water (Boltovskoy et al., 2006; Karatayev et al., 2007), when compared to other invasive bivalves, including *D. polymorpha*. Given their robust tolerance to abiotic stressors, *L. fortunei* may be particularly unlikely to experience mass mortality events. The zebra mussel *D. polymorpha*, on the other hand, has experienced large population declines in recent years, though without clear cut mass mortality events.

Do Salinity Fluctuations Trigger Mass Mortality Events?

For freshwater species expanding into estuarine environments, salinity is an important controller of distributions. It is also highly dynamic, with both regular fluctuations due to tides, as well as human alterations and extreme events. Salinity appears to be particularly important in controlling the distribution of the golden mussel *Limnoperna fortunei* in estuaries (Angonesi et al., 2008). *Corbicula* sp., on the other hand, appear to be tolerant to a wide range of salinities in estuaries, with a higher tolerance of 20 ppt during cooler winter months than during summertime conditions (∼15 ppt) (Ferreira-Rodríguez and Pardo, 2016), though salinity fluctuations in the lower portion of the Minho estuary were hypothesized as a potential contributor to a mortality event during an extreme heat wave and drought in Portugal (Ilarri et al., 2011). Given that salinity can control the distribution of freshwater invasive bivalves, and can vary greatly due to storms, alterations to land use, and even seasonal shifts in river discharge, salinity should be a potential trigger of mass mortality events. However, to date, none have been documented. This topic requires additional study, and our understanding of the role of salinity in controlling the distribution of invasive bivalves lags behind other abiotic factors such as temperature (Feng and Papeş, 2017).

What Role, If Any, Do Disease Outbreaks Play in Mass Mortality Events of Invasive Freshwater Bivalves?

Our understanding of disease induced mass mortality of freshwater bivalves in general is limited, although mass mortality events driven by disease outbreaks have been shown in native unionid mussels (Carella et al., 2016) and marine bivalves such as oysters (Burreson and Ragone, 1996; Lacoste et al., 2001). Invasive species often leave behind their parasites (e.g., Blakeslee et al., 2008, 2012), so parasitic species that act as controls in the native range may simply not be found in the new invasive range. However, genetic diversity of invasive species is often lower than in their native range due to founder effects (Sakai et al., 2001), and this lack of genetic diversity should make them more vulnerable to disease outbreaks. *Corbicula*, as a species that is entirely androgenetic clones in the invasive range in both North America (Lee et al., 2005) and in Europe (Sousa et al., 2007; Gomes et al., 2016), ought to be extremely vulnerable to disease outbreaks, but to our knowledge, none have been demonstrated. There is also the possibility for the transmission of diseases between native and invasive bivalves, though this has not been documented; the transmission of new diseases by invasive species can have substantial negative impacts on native species, however (e.g., Andreou et al., 2012).

How Will Climate Change Alter the Frequency and Magnitude of Mass Mortality Events?

Climate change is predicted to have a wide variety of impacts on invasive species (Hellmann et al., 2008), including altered distributions of existing invasive species. For some invasive bivalves, warming temperatures have opened additional habitats that had previously been unsuitable climatically due to minimum temperatures (McDowell et al., 2014). Expanding into newly suitable habitats may make invasive bivalves vulnerable to occasional extreme cold, leading to mortality events, similar to those documented in the Great Lakes region of the United States (Smith et al., 2018). Within existing populations, higher temperatures could lead to more frequent mortality events during the summer, such as those observed by McDowell et al. (2017). Finally, both drought and flooding were important causes of mortality events for both native and invasive bivalves. Given that climate change is forecasted to increase the intensity of rain events but reduce their frequency (Trenberth, 2011), both drought- and flood-induced mass mortality events are likely to become more common.

Will the Occurrence of Die-Offs and Ecological Impacts be Similar in the Native and Invaded Range?

To our knowledge, no research has documented mass mortality events of invasive bivalve species within their native range, let alone compared the frequency and magnitude of mass mortality events between the native and invaded range. Comparisons of mass mortality events between the two ranges would allow us to determine if massive die-offs are more common in the invaded range than in the native range. In theory, invasive species would be less adapted to the abiotic conditions in their invasive range as they did not evolve there. Because invasive species can undergo rapid evolution, however, invasives may be as well-adapted to a local environment as native species (Oduor et al., 2016). If differences exist between the native and invasive ranges, the impact of invasive bivalve species on communities and ecosystem function may vary dramatically in the invasive range compared to the native. It should be noted, however, that this is currently speculative for invasive freshwater bivalves, but deserves future attention.

Which Organisms Benefit From Mass Mortality Events?

Scavengers ought to exploit the availability of fresh carrion during mortality events, though this has not been established in the literature aside from one report (Mouthon, 2001), perhaps due to the difficulty in capturing transient effects caused by mortality events. For many other taxa the impacts have not been tested or have had inconclusive results. Within terrestrial systems, invertebrates clearly responded to a simulated mortality event, with higher diversity and density that scaled with higher inputs of carrion (Novais et al., 2015). However, a manipulative experiment in a flowing river found no difference in fungi, invertebrates and in decomposition rates (Novais et al., 2017). The responses to mortality events may be highly context dependent, with impacts in lotic systems being less pronounced than those in lentic or terrestrial environments. With inputs into terrestrial systems, there is evidence that mortality events can alter the abundance and biomass of aboveground consumers (Novais et al., 2015), but impacts on belowground communities have yet to be addressed. Further manipulative experiments are needed to fully explore the impacts mortality events have on other communities, in particular to quantify what other organisms are benefitting from carrion inputs.

In addition to trophic benefits, the mass mortality of invasive bivalves may provide competitive release for native species if they are able to survive the trigger of the mortality event, as invasive species often compete with natives. *Corbicula* may compete with native mussels for seston food resource (Leff et al., 1990), and have been shown to lead to lower survival and growth rates of native mussels (Ferreira-Rodríguez et al., 2018) and high densities may reduce the survival of mussel glochidia (Modesto et al., 2019). Zebra mussels compete with native species for both food resources (Baker and Levinton, 2003) and space (Lauer and Spacie, 2004). A mass mortality event of invasive bivalves could lead to higher survival, growth, and reproduction of native bivalves.

How Long Do Effects Remain in the Ecosystem?

Soft tissue from bivalves will be rapidly consumed or decompose but on the other hand, the shells can persist for several decades (see above). Additionally, the shells of *Corbicula* sp. are thicker, and more resistant to decay than those of some native species (Ilarri et al., 2019). For both soft tissue and shells the decay rates are highly context dependent—for example, if shells are deposited in areas with high current velocity the decomposition rate will be very different than if shells are deposited on river banks during floods. Similarly, decomposition rates may also be very distinct in different climatic regimes—in areas with more precipitation, decomposition ought to be much faster than in more arid ecosystems. We need quantitative studies to assess the persistence of the impacts associated with mass mortality events of invasive bivalves. In addition, effects may vary from species to species. For example, bivalves with thicker or harder shells, will in theory, take more time to erode and so persist for more time in the system. Longer term monitoring of locations affected by mass mortality events may be needed to understand longer term impacts, especially within terrestrial systems.

CONCLUSION

Our understanding of the causes and impacts of mass mortality events of invasive freshwater bivalves is developing, though several key questions remain. It remains unclear whether these are "black swan events" (sensu Anderson et al., 2017)—rare events that can have profound impacts on populations and ecosystems—or a regular feature of a community dominated by invasive species. Our ability to answer these questions will be important in creating a better understanding of how mass mortality events of invasive bivalve currently affect aquatic ecosystems, and how they will do so in the face of continued biotic homogenization and climate change. Because invasive bivalves are well-suited for manipulative studies, this faunal group can be used to further understand the trophic and non-trophic consequences of massive mortalities in aquatic ecosystems.

AUTHOR CONTRIBUTIONS

WM conceived of this study. WM and RS contributed to all other components of this study.

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Fish, Including Their Carcasses, Are Net Nutrient Sources to the Water Column of a Eutrophic Lake

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Animals can act as sources or sinks of nutrients in ecosystems, and their role in this context may depend on the fate of nutrients in decomposing carcasses, which may contain recalcitrant structures such as bones. Our goal was to assess whether a fish population with high biomass is a source or sink of nutrients to the pelagic zone of a eutrophic lake over time scales ranging from days to 20 years. We developed a population-level model based on a 20-year (1996–2015) dataset for gizzard shad (*Dorosoma cepedianum*) in Acton Lake, a eutrophic reservoir in southwest Ohio, U.S.A. In addition, we used data from experiments that quantified nutrient mineralization rates from carcasses as functions of fish size and temperature. Nitrogen (N) and phosphorus (P) remineralization rates from carcasses increased with temperature and decreased with fish size. Over the 20 years, almost all (~99%) of the nitrogen (N) and phosphorus (P) produced as gizzard shad carcasses was remineralized back to the water column. At the ecosystem scale, carcass nutrient dynamics followed a seasonal pattern, with a net accumulation of carcass nutrients in winter but a net depletion of the carcass nutrient pool in summer, due to mineralization. Dynamics of carcass production and remineralization were strongly influenced by young-of-year fish (YOY), for both N and P, because the number of fish born varied considerably across years, YOY have high mortality rates, and YOY carcasses decompose rapidly. On an annual basis, in a few years biomass production was higher than mineralization, suggesting that in these years fish biomass may act as a nutrient sink at the annual scale. However, nutrient excretion by the population greatly exceeded sequestration of nutrients in biomass (living and dead). Because most of the nutrients consumed (and excreted) by this population are derived from the benthos, at the lake-wide scale and considering all fluxes, the population is a significant net source of nutrients to the pelagic habitat. Our model demonstrates the relevance of considering spatial and temporal scale as well as long-term population dynamics when studying the role of animals as nutrient sources or sinks.

Keywords: nutrient cycling, decomposition, nitrogen, phosphorus, mineralization

INTRODUCTION

Animal populations can be nutrient sources or sinks (Kitchell et al., 1975; Beasley et al., 2012; Barton et al., 2013; Atkinson et al., 2017; Subalusky and Post, 2018). In aquatic ecosystems, an animal population can act as a source of nutrients when releasing nutrients in available forms to other members of the ecosystem, and as a sink when removing nutrients from circulation in the ecosystem (**Figure 1**). Studies on nutrient cycling by fish (and other aquatic animals) have focused mostly on their role as a nutrient source through excretion (Atkinson et al., 2017; Subalusky and Post, 2018). However, because fish can represent a large proportion of animal biomass in many ecosystems (Barton et al., 2019), because they are long-lived compared to other organisms, and because their bodies contain recalcitrant tissues like bones and scales, it has been suggested that fish populations act as nutrient sinks in pelagic freshwaters (Kitchell et al., 1975; Sereda et al., 2008). The main ways in which a fish population can be a nutrient sink are (1) if biomass increases, i.e., when growth and reproduction exceed mortality, (2) if emigration from the ecosystem exceeds immigration to that ecosystem, and (3) if nutrients stored in carcasses are not mineralized back to the water column, but rather remain stored in sediments in a recalcitrant form for a long time (Vanni et al., 2013).

If animal biomass is relatively stable, the fate of carcasses may be especially important in determining whether animals are nutrient sources or sinks. In general, animal carcasses are nutrient-rich resources that generally decompose much faster than plant detritus (Barton et al., 2013, Benbow et al., 2019). However, vertebrate carcasses contain bone, a phosphorus (P)-rich component that decomposes much more slowly than other tissues (e.g., Parmenter and Lamarra, 1991; Subalusky et al., 2017). Thus, carcasses could represent a relatively long-term P sink, and if P is the limiting nutrient this could constrain primary production. In addition, carcasses are produced and deposited at various spatial and temporal scales, which can modulate their role as sources or sinks. For example, in some populations carcasses are produced in highly episodic mass mortality events, whereas in other populations mortality is more temporally constant or varies seasonally (Fey et al., 2015; Subalusky et al., 2017). Carcass deposition can also be spatially variable, producing nutrient hotspots in the landscape that influence soil, or sediment biogeochemistry and primary production (Bump et al., 2009; Keenan et al., 2018). More specifically, in aquatic ecosystems, carcasses of pelagic animals may sink to the sediments, out of the euphotic zone and away from pelagic primary producers; thus they can be a sink for the pelagic ecosystem, but whether they are depends on the rate at which carcass nutrients are remineralized and transported to pelagic primary producers (Beasley et al., 2012; Vanni et al., 2013).

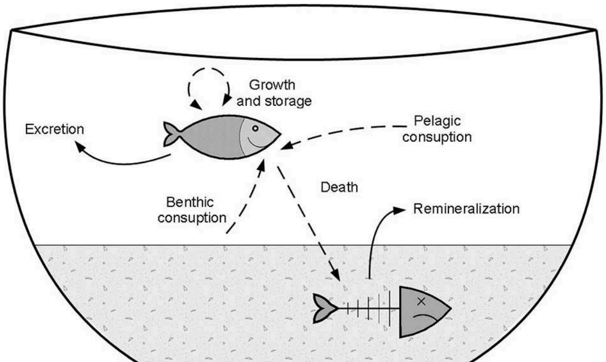

FIGURE 1 | Conceptual model showing the fluxes of nutrients derived from fish (sources to the water column, solid arrows) and stored in fish biomass (sinks from the water column, dashed arrows). Fish can act as nutrient sinks when they remove nutrients from circulation and store them in their bodies for growth, or when fish die and nutrients are stored in recalcitrant tissues. On the other hand, they act as sources of nutrients to the water column when they are releasing nutrients through excretion and remineralization of nutrients stored in carcasses. Piscivory is omitted here for simplicity.

In this paper, we assess whether a fish population with high biomass and growth rate is a source or sink of nitrogen and phosphorus to the pelagic zone of a eutrophic lake, at various time scales ranging from days to years. To accomplish this, we used a population-level model for gizzard shad (*Dorosoma cepedianum*) over a 20 year period in Acton Lake, a eutrophic reservoir in Southwest Ohio, U.S.A. Gizzard shad are abundant in lakes and reservoirs of the Midwest and southeast USA, and non-larval age classes feed mostly on organic detritus associated with sediments (Schaus et al., 1997; Higgins et al., 2006). Consumption of benthic-derived nutrients and subsequent excretion of these nutrients into the water column by gizzard shad represents an important nutrient source to phytoplankton (Shostell and Bukaveckas, 2004; Vanni et al., 2006; Schaus et al., 2010; Williamson et al., 2018). However, the role of nutrient storage in living fish and the fate of nutrients in carcasses is not well-known at the ecosystem scale. Our approach is comprehensive in that we explicitly quantified how this population can be a nutrient sink (storage in living biomass and carcasses) as well as a source (excretion, remineralization from carcasses) to pelagic primary producers, incorporating all life stages (including larvae) over a 20-year period.

METHODS

Population Model

We used the gizzard shad population model of Williamson et al. (2018), which estimated fish population size and age structure for multiple cohorts over a 20-year period (1996–2015). The model combines data from electrofishing, hydroacoustics, and larval fish sampling, to track the number and size of fish in each age class, and ultimately the mass of nutrients sequestered in living biomass and produced as carcasses, on a daily basis. Details of the population model are described in Williamson et al. (2018). Briefly, the number of larval fish was estimated using weekly tows with an ichthyoplankton or neuston net during the period of larval production (generally, May-June), for all 20 years. This allowed us to estimate the number of fish born each year, following methods of Bremigan and Stein (2001).

Non-larval fish were sampled each August (1999–2015) using hydroacoustics (Hale et al., 2008), which allowed us to annually estimate population size and age-structure. We do not have hydroacoustics estimates for 1996–1998; to obtain population estimates in August of each of these 3 years, we used a regression between electrofishing data (catch per unit effort, CPE) and hydroacoustics estimates developed for years in which we have both types of data (Williamson et al., 2018). Thus, for young-of-year (YOY) fish, we had estimates of cohort density (no. YOY fish per ha) during the hatching period (May–June) and in August, and for non-YOY fish we had estimates each August. The number of fish present in each cohort (ages 0, 1, 2, 3, and 4+ years) on each day in between population estimates was interpolated using daily instantaneous mortality rates, as described in Williamson et al. (2018). Fish size (wet mass) was obtained from larval fish tows, hydroacoustics and electrofishing, and interpolated on a daily basis (Williamson et al., 2018). Mortality, i.e., the number of fish in each cohort dying each day, was estimated by difference in cohort density on successive dates; this assumes no immigration or emigration in this population. We assumed that piscivory accounted for 15% of the mortality of gizzard shad smaller than 200 mm total length, when temperature was $> 10^{\circ}$C; for fish $>$200 mm, and for all fish when temperature was $<10^{\circ}$, we assumed no piscivory. These assumptions are based on piscivory estimates for largemouth bass and other piscivores in Acton Lake (Aman, 2007). Fish that died but were not consumed by piscivores were assumed to sink to the sediments as carcasses.

Nutrient Pools and Fluxes

Using data on the number and size of fish in each cohort, we estimated the mass of nutrients (N and P) in live fish biomass, and "lost" from the pelagic zone due to mortality on a daily basis. We also considered nutrients excreted by fish in the water column, using data from Williamson et al. (2018).

For each cohort, we modeled pools of carcass N and P as the balance of daily carcass production (mortality not due to piscivory) vs. mineralization. For each cohort, carcass production (no. carcasses produced ha^{-1} d^{-1}) was converted to nutrient mass produced as carcasses (g N and P ha^{-1} d^{-1}). To do so, fish wet mass was first converted to dry mass, and then to N and P mass using size-specific data derived from the Acton Lake gizzard shad population (Schaus et al., 1997; Pilati and Vanni, 2007; Torres and Vanni, 2007).

Remineralization of nutrients from gizzard shad carcasses was estimated using data from lab experiments that quantified nutrient mineralization rates from carcasses of different sizes, incubated at three temperatures (5, 15, and 25°C). Details on these experiments can be found in the **Supplemental Material**; briefly, we incubated YOY and adult carcasses at these three temperatures and measured their nutrient contents on several dates for up to 100 days (or shorter if carcasses fully decomposed). Based on these experiments, we calculated the decay rate (k) of carcass N and P by fitting the equation $M_t = M_0e^{-kt}$, where M_t and M_0 are the masses of the element (N or P) remaining in carcasses at time t (days) and k is the instantaneous daily decay rate. Then we used two-way analysis of variance (ANOVA) to evaluate the individual and interactive effects of fish size and temperature on decay rates (k) for carcass N and P. When ANOVA detected significant effects of the experimental factors, we used Tukey's post-test for multiple comparisons to discriminate significant statistical differences between levels of experimental factors.

To estimate daily mineralization rates as a function of lake temperature, for each fish size class, we used Q_{10}, defined as the factor by which a decay rate increased when temperature increased by 10°C. This factor is commonly used in studies testing the temperature effects on biological rates (Lloyd and Taylor, 1994; Downs et al., 2008). For example, if a rate doubles when temperature is increased from 5 to 15°, the $Q_{10} = 2$. The values of Q_{10} for N and P decay rates for YOY and adult carcasses were estimated by using the k values computed previously; we calculated separate Q_{10} values for 5–15° and 15–25°. Q_{10} values for adult carcasses were used for all non YOY age classes. Cohort carcass mineralization rates were summed to obtain carcass mineralization rates at the lake-wide scale. We assumed that all nutrients mineralized from carcasses were returned to the water column in dissolved inorganic form and therefore were a nutrient source to phytoplankton; i.e., we assumed no benthic uptake of nutrients remineralized from carcasses. We feel that this is a valid assumption because Acton Lake is turbid and very little light reaches sediments (Secchi depth usually $\sim$0.5–0.7 m); therefore, uptake by benthic algae is minimal, although we acknowledge that some remineralized nutrients could be taken up, at least temporarily, by sediment microbes.

To estimate the flux of nutrients through piscivores, we assumed that piscivore biomass was constant at the lake-wide scale. We assumed that piscivores assimilated 80% of the N and P they ingested from gizzard shad bodies, and therefore egested 20% of ingested N and P as feces (Schindler and Eby, 1997). We assumed that piscivore gross growth efficiency (N or P growth/N or P ingestion) was 30% for both N and P (based on Schindler and Eby, 1997). Therefore, piscivores excreted 50% of the N and P they consumed; this excretion was considered a source to the water column. Because we assumed that piscivore population biomass was constant, the production of piscivore carcasses was considered to be 30% of ingested N and P, i.e., equal to their growth at the population level. Thus, nutrients consumed by piscivores were allocated as follows: 20% egested as feces; 30% to mortality (piscivore carcasses), and 50% excreted as dissolved nutrients. N and P in piscivore carcasses was assumed to be mineralized at rates equal to those for adult gizzard shad carcasses. N and P egested by piscivores as feces was assumed to remineralize at the same rate as YOY gizzard shad carcasses, i.e., faster than adult shad or piscivore carcasses.

The modeling approach we employed for the fate of nutrients through piscivores is obviously a simplification, although assumptions about piscivory are based on data from Acton Lake. Nevertheless, we explored additional piscivory scenarios in which we relaxed these assumptions and compared these simulations to our "baseline" model. Specifically, we explored additional scenarios regarding our assumption that piscivore biomass was constant, and that piscivory accounted for 15% of mortality of gizzard shad $\leq$ 200 mm when temperature was $\geq$

10°. Data from Acton Lake show that piscivore catch-per-unit-effort declined in Acton Lake from 2003/2004 to about 2008, but no data are available before 2003 (**Supplemental Material**). We considered a scenario in which piscivore population biomass doubled from 1998 to 2003, from 20 to 40 kg wet mass/ha, to mirror the decline over the following 5 years. In addition, we explored scenarios in which we varied the percentage of shad mortality caused by piscivory, from 10 to 30% (see **Supplemental Material** for details), deviating from the baseline model in which it was 15%. In all scenarios, the flux of nutrients through piscivores was relatively small compared to that via non-piscivory gizzard shad mortality (see Results). Because the contribution of piscivores to the flux of nutrients was small, and for model simplification purposes, here we present the results that include the flux of nutrients through piscivory, i.e., piscivores excretion and mineralization from piscivores feces and carcasses, combined with non-piscivory gizzard shad mortality, i.e., carcass mineralization.

RESULTS

Remineralization rates of carcass nutrients (k) increased with temperature and were higher for YOY than adults (**Figures 2**, **3**, **Supplemental Table 2**). k was higher for N than P, reflecting the greater recalcitrance of P because of bones and scales. Q_{10} values for N and P ranged from ∼1 to 3 depending on fish size and temperature increment (5–15 or 15–25°) (**Table 1**).

Over the 20-year period, 99.4% of the N and 98.6% of the P produced as gizzard shad carcasses were remineralized back to the water column. The dynamics of carcass production and remineralization followed a seasonal pattern and were strongly influenced by young-of-year fish (YOY), for both N and P (**Figure 4**). Nutrients accumulated in the carcass pool over winter months, when mortality was high and mineralization rates were low; in contrast, a net depletion of the carcass nutrient pool occurred during warmer months when mineralization rates were high. During July and August, a small peak in the carcass nutrient pool was also detected, due to a short-term accumulation of carcasses of YOY fish, followed by a rapid decrease until October (**Figure 4**). These dynamics reflect the high mortality rates, and rapid carcass mineralization rates, of YOY fish.

On an annual basis, N and P sequestered by the gizzard shad population via biomass production was higher than carcass mineralization in a few years, suggesting that fish biomass (living and dead) may act as a nutrient sink on a yearly time scale (**Figure 5**). However, in most years mineralization exceeded biomass production. Furthermore, for both N and P, excretion rates were higher (often much higher) than biomass production

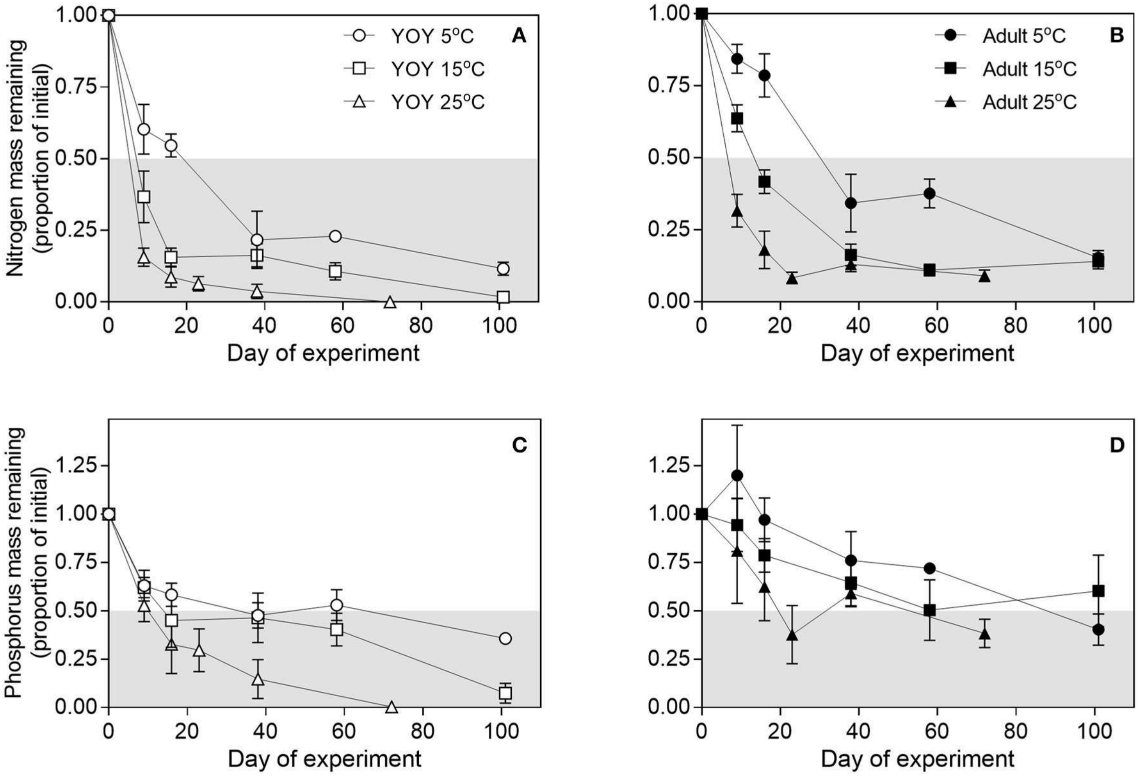

FIGURE 2 | Individual and interactive effects of fish size and temperature on patterns of mass remaining for **(A,B)** nitrogen **(C,D)** phosphorus mass in decomposing fish carcasses throughout time. Values are expressed as the proportion of initial mass in carcasses. Data for young-the-year (YOY) and adult fish are shown in separate panels for clarity. The transition between the white and gray areas in the panels indicate the half-lives of mass decay. Data points are mean ($n = 3$) and error bars are ± 1SEM.

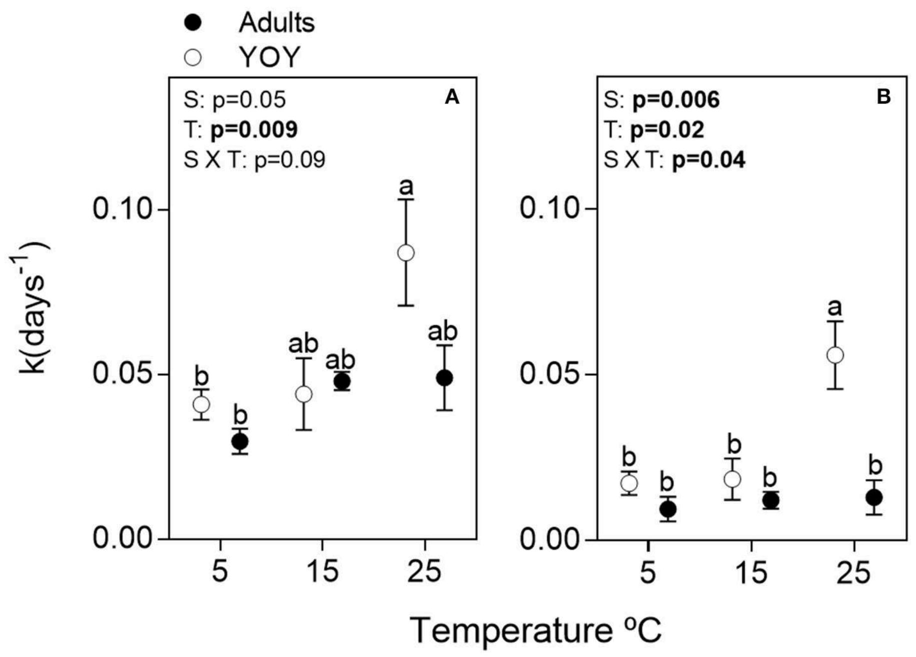

FIGURE 3 | Individual and interactive effects of fish size (S) and temperature (T) on time-integrated fish carcass decay coefficients (k) for **(A)** nitrogen and **(B)** phosphorus. Data points are means ($n = 3$) and error bars are ± 1SE. Different letters above treatments indicate significant statistical differences among treatments. Bold *p*-values depict significant statistical effects (Tukey's *pos-hoc* test; $p < 0.05$).

TABLE 1 | Q_{10} values for N and P decay rates through 38 days of the laboratory experiment.

Size class	Temperature range	Nitrogen	Phosphorus
YOY	5–15°	1.068	1.069
	15–25°	1.992	3.023
Adult	5–15°	1.606	1.278
	15–25°	1.020	1.074

Q_{10} values were calculated as the ratio between time-integrated fish carcass decaying coefficients (k) for each temperature level.

in all years (**Figure 5**). Thus, considering all fluxes, this fish population is a net source of N and P to the water column on an annual basis.

On a daily basis, the gizzard shad population was usually a source of nutrients. Thus, over the study period, mineralization plus excretion exceeded carcass production on 92.6 and 86.8% of days for N and P, respectively. The days on which nutrient accumulation in carcasses was higher than mineralization plus excretion occurred only during winter.

Piscivory accounted for the consumption of 15,133 and 4,037 g P/ha, representing, respectively, only 5.5 and 5.1% of total gizzard shad N and P biomass "lost" to total mortality. Over the 20-year period, almost all nutrients (99.9% of N and 99.7% of P) consumed by piscivores were released back to the water column via excretion, egestion, and piscivore carcass decomposition.

Results of the piscivory scenarios showed that the ultimate fates of nutrients from gizzard shad mortality were relatively insensitive to the assumptions in the baseline model. Doubling piscivore biomass from 1998 to 2003 reduced the flux of dissolved nutrients through piscivores (via piscivore carcass and feces mineralization, plus piscivore excretion) by 13% (N) or 15% (P) over this 5-year period, as these nutrients were stored in living piscivore biomass rather than mineralized (**Supplemental Material**). However, in total all of these fluxes through piscivores represent <6% of the flux via mineralization of gizzard shad carcasses; therefore, doubling piscivore biomass reduced total fluxes of N and P to the water column by <1% compared to the baseline model. Varying the percentage of gizzard shad mortality attributed to piscivores also had a relatively small effect on net N and P fluxes to the water column. As this percentage increased from 10 to 30%, N and P mineralization from the gizzard shad carcass pool declined, but this was accompanied by compensatory increases in piscivore excretion plus the mineralization of piscivore carcasses and feces (**Supplemental Figure 6**). In all scenarios, the percentage of nutrients produced via shad mortality that remained as carcasses (shad plus piscivores) was <0.6% for N and <1.4% for P. Thus, in all scenarios >99% of N and >98% of P were returned to the water column.

DISCUSSION

Our model suggests that, considering all fluxes of nutrients, this fish population is a net source of N and P to the water column, even though fish biomass is relatively high and fish are

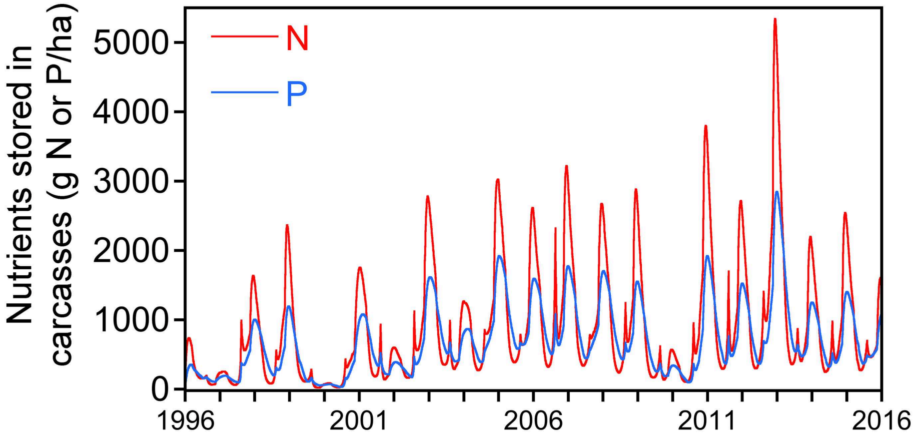

FIGURE 4 | Twenty-year time series showing the dynamics of phosphorus (blue line) and nitrogen (red line) stored in carcasses over time. The large peak occurring during winter is due to high mortality (high carcass production) rate of all age classes during this time, whereas the smaller peaks occurring in late July-August are due to production of carcasses from high mortality of young-of-year fish.

much more long-lived compared to phytoplankton, which are the dominant primary producers. In Acton Lake, phosphorus "trapped" in live gizzard shad biomass averaged about 47 μg P L^{-1} over our 20 year study, and lake "total P" (P in the water column, excluding fish) averaged about 105 μg P L^{-1} Therefore, the gizzard shad population contains a pool of P equal to roughly 45% of the traditionally-measured "total P." However, almost all of this P becomes available relatively quickly after fish die and decompose. Thus, neither the high biomass of living fish nor the production of carcasses renders this population a long-term nutrient sink.

The rate at which nutrients are remineralized from fish carcasses is likely to vary greatly among ecosystems. Boros et al. (2015) argued that fish carcasses are not likely to act as a long-term P sink in warm-temperate shallow lakes. In their experimental work, carcasses of gizzard shad and bluegill (*Lepomis macrochirus*) decomposed completely over ∼3 months in mesocosms that were warm (mean temperature 24.5°C) and well oxygenated, i.e., conditions similar to those in Acton Lake in summer. Thus, Acton Lake has conditions that are favorable for carcass decomposition. In deeper, colder lakes fish carcasses may be nutrient sinks (Kitchell et al., 1975; Parmenter and Lamarra, 1991), but much depends on carcass remineralization rates. Parmenter and Lamarra (1991) studied carcass decomposition of rainbow trout (*Oncorhynchus mykiss*) in colder environments (2–5°C) and found that most of the decomposition activity took place during the initial 60 days of carcass decomposition. By the end of the experiment fish carrion lost 95% of original carcass N, but only 60% of carcass P due to its presence in more recalcitrant tissues. Chidami and Amyot (2008) argued that water temperature can be used as a surrogate for decomposition rates in freshwaters. Studying boreal lakes, they found that decreasing temperatures are related to increased decomposition half-lives, probably due to lower metabolic activity of decomposing bacteria and scavengers.

Animal size also plays a role. We found that remineralization rates were higher for small gizzard shad carcasses than for large carcasses, although all sizes decomposed rapidly at warm temperatures. Very large animal carcasses will probably decompose even more slowly; for example, Subalusky et al. (2017) found that wildebeest bones take 7 years to fully decompose even in a warm, tropical river. In ecosystems where bones mineralize slowly, carcasses are more likely to be long-term sinks for P, than for N.

In our model, carcasses mineralized fairly rapidly, so at an annual scale they were not a sink for nutrients. However, because of variation in water temperature and fish size, we found temporal variation in the amount of nutrients being stored in vs. mineralized from carcasses, at annual and seasonal scales. Thus, while the population is a net source of N and P to the water column, there are specific periods when N and P accumulate in live biomass or in carcasses, and during these periods the population acts as a temporary sink. During winter, for example, both excretion and decomposition rates are low, promoting a short-term nutrient accumulation in fish carcasses. In terms of fish population biomass, short term sinks also occur in summer during the period of YOY growth followed by higher rates of YOY mortality. The strong influence of the YOY on the dynamics of carcass production and N and P remineralization (**Supplemental Figure 4**) is due to the variability in the number of fish born across years (Kraft, 1992; Williamson et al., 2018), the high mortality rates of this age class, and the fact that YOY carcasses decompose rapidly. However, during these periods excretion rates are high and exceed this potential sink. These dynamics illustrate the importance of explicitly considering both temporal scale and the multiplicity of potential fluxes of nutrients when analyzing the extent to which fish are sources or sinks of nutrients.

It is potentially informative to compare nutrient fluxes from our model with other fluxes from sediments to water. Nowlin

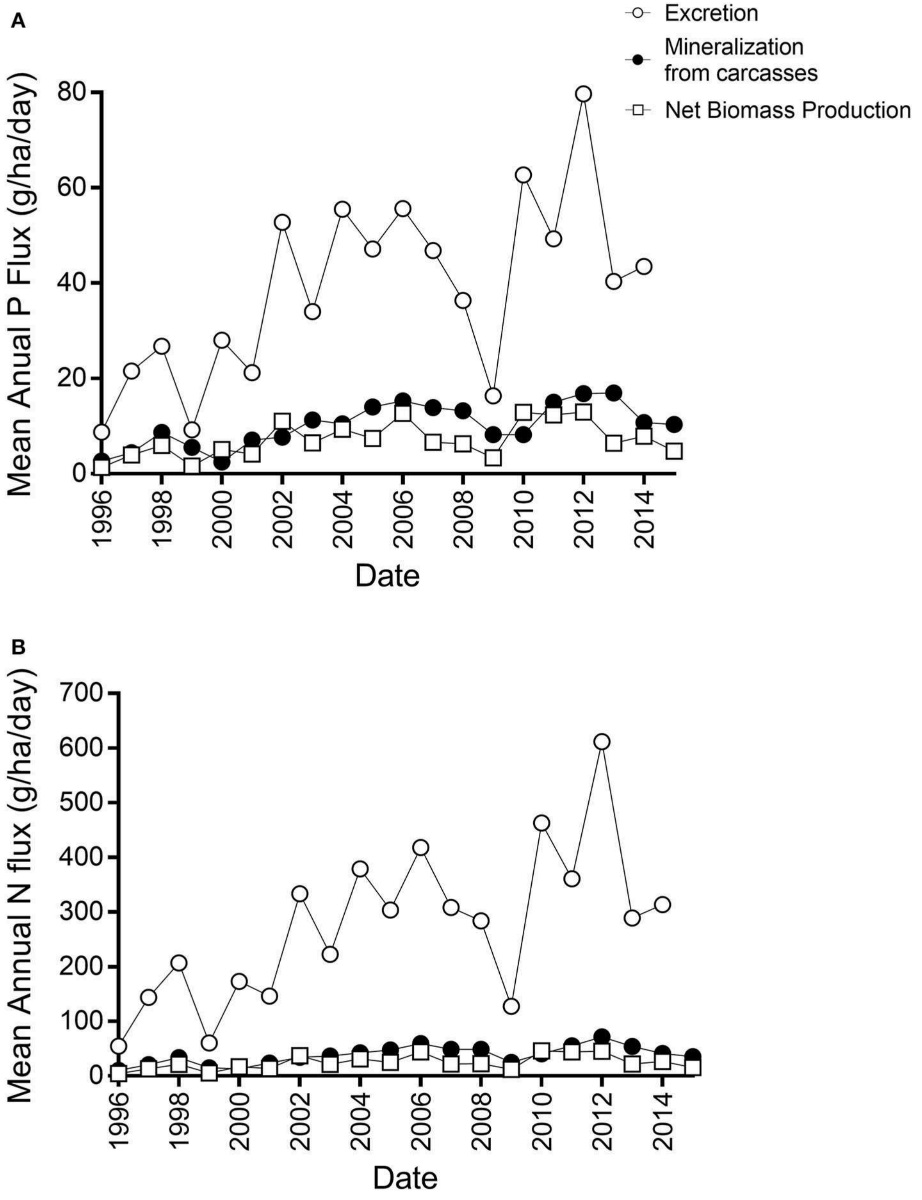

FIGURE 5 | Annual fluxes of phosphorus **(A)** and nitrogen **(B)** for net biomass production (white square), carcass remineralization (black dots), and excretion (white dots).

et al. (2005) measured N and P fluxes from Acton Lake sediments using sediment core incubations; these "direct" fluxes are probably mediated mainly by microbes but also may include excretion by small invertebrates that happen to be in the cores. In summer (the only period when core incubations were done), direct fluxes of NH_4 exceed carcass N mineralization by 4-5X at the lake-wide scale (**Table 2**). For P, the results are more complex. As would be expected, direct flux of P is much higher (>9X) from anoxic, hypolimnetic sediments than from oxic sediments; the mineralization rate of P from carcasses is similar in magnitude to direct flux of P from oxic sediments (**Table 2**). At a lake-wide scale, accounting for the areas of oxic and anoxic sediments, direct P flux exceeds carcass mineralization by 4-5X. However, Nowlin et al. (2005) showed that very little of the P released from hypolimnetic sediments is transported to the euphotic zone where phytoplankton can use the P, probably because P precipitates with calcium in the hypolimnion and sinks to sediments. Regardless of the relative magnitudes of direct fluxes vs. carcass mineralization rates, it is clear from our study that carcasses do not represent long-term sinks. Furthermore, during summer, excretion by gizzard shad exceeds direct fluxes from sediments for both N and P (**Table 2**).

Our study is unique in that we have extensive data on the fish population dynamics (including all age classes) at the

TABLE 2 | Comparison of different fluxes from sediments to water in Acton Lake.

	Mean N flux ($g N ha^{-1} d^{-1}$)		Mean P flux ($g P ha^{-1} d^{-1}$)	
Nutrient source	**Summer**	**Annual**	**Summer**	**Annual**
Excretion by gizzard shad	622.0	262.3	97.1	37.7
Mineralization of gizzard shad carcasses	31.5	35.7	8.4	10.2
Direct flux from sediments				
Outflow site (anoxic, hypolimnion)	194.0		91.1	
Inflow site (oxic, unstratified)	117.6		9.8	
Lake-wide (weighted average)	144.3		38.2	

Summer values are those from July through September, and annual values are over the entire year. Excretion data are taken from Williamson et al. (2018); the "annual" mean presented here is the mean of the points in ***Figure 5****. Direct flux data are taken from Nowlin et al. (2005) and are based on data from summer 1996, the only time period for which we have experimental data. Fluxes were measured by incubating sediment cores from two sites, an Inflow ("River") site where depth is ∼1 m and the water column is always well-mixed and oxic, and an Outflow ("Dam") site where sediments were taken from the anoxic hypolimnion. Details are provided in Nowlin et al. (2005). The lake-wide direct flux value was obtained as a weighted mean assuming that anoxic and oxic sediments comprise 35 and 65% of the lake bottom, respectively.*

ecosystem level and over a 20-year period. This large and comprehensive dataset allowed us to produce robust estimates for population dynamics as well as fluxes of nutrients mediated by the population. With the exception of research on Pacific salmon (e.g., Tiegs et al., 2011; Rüegg et al., 2014), the fate of carcasses in freshwater environments has not been explored as much as in marine and terrestrial systems (Beasley et al., 2012), so our study helps to fill this gap in the nutrient cycling literature. We encourage additional whole-ecosystem studies that assess whether animals are nutrient sources or sinks. The fates of nutrients associated with different mortality sources need to be explored more (Barton et al., 2019). Based on our results, nutrients consumed by fish piscivores are not likely to be long-term sinks. However, piscivorous birds may function as sinks by transporting nutrients away from an aquatic ecosystem. In addition, the role of scavengers in processing carcass nutrients needs to be evaluated. More broadly, to fully understand the role of animals as nutrient sources or sinks, we need studies in a range of environments that reflects the breath of variation in habitat features such as depth, temperature, the balance of benthic vs. pelagic production, and other potential drivers.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Miami University Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

RN and MV conceived the population model. LC and SP conducted the lab experiments. MG and MV collected the data on the fish population. RN, MV, and LC wrote the final versions of the manuscript. All authors contributed to writing the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00340/full#supplementary-material

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Microbial Community Response to a Novel Salmon Resource Subsidy

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Salmon decomposition is traditionally viewed through the lens of energy and nutrient subsidies, but not as a potential "microbial subsidy." Microbial communities residing on and within spawning salmon are directly introduced into streams after host death. This incorporation takes the form of microbes sloughing off and integrating into substrate biofilms, or indirectly, by macroinvertebrates facilitating dispersal via consumption. The objective of this study was to determine the effects of salmon carcass-derived microbial communities on stream biofilms and macroinvertebrates during an experimental salmon carcass addition in a naïve stream (i.e., no evolutionary history of salmon). Microbial communities [epilithic biofilms and within macroinvertebrates (internal)] were sampled at treatment and control sites before (September), during (October), and after (November to following August) a salmon carcass subsidy introduction in 2 successive years (September 2014-August 2016). We found a significant interaction between carcass addition and time on microbial and macroinvertebrate communities. *Heptagenia* (Heptageniidae: grazer) density was five times higher in the salmon reach compared to the control. In the salmon reach during year one, Stramenopiles (i.e., eukaryotic microbes) decreased in biofilm communities after 2 weeks of decomposition. The internal microbiome of *Stegopterna mutata* (Simuliidae: collector-filterer) varied between years but was significantly different between reaches over time during year two of the study, with four times greater abundance of melanogenesis functional pathways (function determined *in silico*) in the control reach. Although unique microbial taxa, introduced to this naïve stream via salmon carrion, persisted in biofilms on benthic substrate and internal to insects during both years, those taxa represented <2% of the relative abundance in microbial communities. These results highlight the importance of allochthonous carrion resources in the microbial ecology of lotic biofilms and macroinvertebrates. Furthermore, this study contributes to previous research into the complex interkingdom interactions in stream communities in response to a novel allochthonous resource.

Keywords: salmon carcass, decomposition, allochthonous resources, community ecology, insect-microbe interactions

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INTRODUCTION

Headwater streams are highly reliant on allochthonous organic matter as an energy base for consumers. Shading from riparian trees restricts the amount of sunlight to most headwater streams thereby limiting autochthonous primary production (Vannote et al., 1980). Therefore, stream trophic networks rely on organic matter decomposition from outside sources. Organic matter decomposition has traditionally been viewed through the lens of carbon and nutrient subsidies (e.g., leaf litter) into the system in ways that alter macrobenthic communities (Polis and Strong, 1996; Hagen et al., 2012; Benbow et al., 2018). Allochthonous organic matter may also act as a "microbial subsidy" source to streams, by transferring novel microbes from one ecosystem to another (Steffan et al., 2017; **Figure 1**). Due to the high diversity of microbes on Earth, each allochthonous resource has an individual microbial community residing on and within it (Lindström and Langenheder, 2012; Locey and Lennon, 2016; Thompson et al., 2017). These novel microbes are hypothesized to be introduced into streams through the addition and transport of the allochthonous resources from adjacent or upstream habitats (e.g., riparian zones or tributaries) (Ruiz-González et al., 2015), but energy, nutrients, and microbes can also arrive in the form of decomposing heterotrophic biomass, such as carrion (Pechal and Benbow, 2016; Benbow et al., 2018).

Macroinvertebrate consumers may directly ingest and subsequently disperse allochthonous resource microbes throughout a stream (McEwen and Leff, 2001). Insects are vectors of microbes from diverse substrates involved in decomposition, transferring the microbes that come into contact from one environment to another [e.g., insects on food: (Blazar et al., 2011); blow flies (Diptera: Calliphoridae) on agar: (Junqueira et al., 2017)]. For example, in a microcosm experiment, dispersal of marked microbes by mayfly (*Baetis* sp.), stonefly (*Pteronarcys* sp.), dragonfly (Aeshnidae) nymphs, and glass shrimp (*Palaeomonetes* sp.), exhibited considerable variation in the abundance of macroinvertebrate-associated bacteria transferred to other surfaces (Leff et al., 1994). In some trials, there was a large pulse of marked microbes dispersed, and in others, no marked microbes were detected. It remains unknown how these occasional releases of macroinvertebrate-associated bacteria ultimately influence microbial structure on downstream surfaces. Stream macroinvertebrates also act as important consumers of biofilms and can alter microbial community structure by selectively consuming taxa (Mulholland et al., 1991; Feminella and Hawkins, 1995; Rosemond et al., 2000; Lang et al., 2015). Alternatively, microbes may slough off the host resource and become integrated into the water column and benthic biofilm microbial communities (Leff et al., 1998; Crump et al., 2012). These changes to microbial communities alter the functional base of the aquatic food web, which may have far reaching effects throughout the stream network (Hall and Meyer, 1998; Meyer et al., 2007). Therefore, it is important to understand how microbial subsidies associated with allochthonous resources influence stream communities, from riparian leaf litter to carrion generated through mass mortalities, such as annual salmon runs.

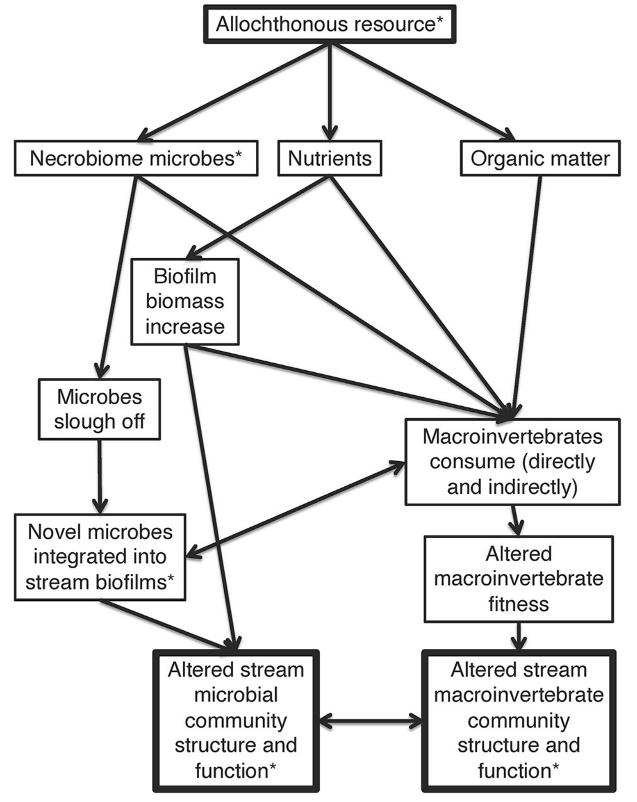

FIGURE 1 | Conceptual framework of allochthonous resources altering microbial and macroinvertebrate communities via nutrients, organic matter, and microbes. Arrows represent directional links of effects. An asterisk represents factors directly measured in this study, while those without asterisks are hypothesized. Hypotheses on the overall importance of each linked agent are not offered, as these may vary over time and space, and the overall figure represents how these components fit into the larger ecosystem context.

Pacific salmon (*Oncorhynchus* spp.) are an important annual input of allochthonous nutrients and organic matter into streams. Particularly in the Pacific Northwest and Alaska, which have a several thousand year long history of native salmon, and other watersheds in North America where they have been anthropogenically introduced as recreational and economic fisheries (Cederholm et al., 1999; Gende et al., 2002; Moore et al., 2004). Benthic biofilms increase in biomass and have lower nutrient limitation when salmon carrion is present (Wipfli et al., 1998; Johnston et al., 2004; Mitchell and Lamberti, 2005; Rüegg et al., 2011). Similarly, macroinvertebrate community structure, function, and growth rates are influenced by decomposing salmon organic matter (Chaloner and Wipfli, 2002; Chaloner et al., 2002; Lessard and Merritt, 2006), often with contrasting effects within different regions and streams (Bilby et al., 1996; Claeson et al., 2006; Janetski et al., 2009, 2013). Temporal dynamics are important in mediating the influence of carcass additions. For example, peak salmon derived nutrient enrichment (determined by stable isotopes) in grazers occurred 1–2 months after carcass introduction, while it occurred 2–3 months after introduction in predatory macroinvertebrates

(Morley et al., 2016). Microbes associated with salmon carcasses were detected in aquatic macroinvertebrates located within Alaskan salmon-bearing streams (Pechal and Benbow, 2016), which demonstrates the potential for salmon carcasses to act as conduits for new microbe introduction into streams. Yet, this potential has not been tested in streams without a historical salmon run, such as those found in the Laurentian Great Lakes watershed, where Pacific salmon have been introduced.

Since first introduced to the Great Lakes region in 1966–1970 to control invasive alewife (Parsons, 1973), chinook (*O. tshawytscha*) and coho (*O. kisutch*) salmon have been naturalized to many tributary streams of the watersheds where they now spawn and die. Therefore, salmon carrion has been a non-native resource subsidy to some Michigan streams for only the last 50 years. After spawning and death, the resulting carcasses may structurally and functionally impact the aquatic communities residing in naïve headwater streams (Cederholm et al., 1989). These annual pulses of salmon increase the nutrients and organic matter inputs to recipient streams, which can have far reaching effects on both aquatic and terrestrial ecosystems throughout the food web (Schuldt and Hershey, 1995; Bilby et al., 1996). The salmon resource may be directly used by both stream macroinvertebrates and microbes, as well as indirectly through nutrient and dissolved organic matter subsidy pathways (Collins et al., 2011; Levi and Tank, 2013; Levi et al., 2013). However, salmon do not migrate to all Michigan streams, such as those with dams, providing an opportunity to investigate salmon carrion effects on microbial and macroinvertebrate communities in historically naïve systems through carrion subsidy introduction and monitoring.

The objective of this study was to evaluate allochthonous salmon carcass resource subsidy effects on aquatic macroinvertebrate and microbial communities in a naïve Michigan stream. We postulated these communities would demonstrate short- and long-term responses to introduced salmon carcasses. Specifically, we predicted in carcass-introduced habitats that: (1) macroinvertebrate communities would be initially dominated by shredders and transition to an increase in grazers and collectors; (2) biofilm communities would be dominated by heterotrophic bacteria compared to the control habitats; (3) the internal macroinvertebrate microbiomes would be supplemented with salmon carcass-associated microbes after carcass introduction; and (4) salmon carcasses would introduce microbes to the stream, some of which would persist and become more abundant, while others diminish.

MATERIALS AND METHODS

Experimental Design

This study was conducted in Hunt Creek on the property of the Hunt Creek Fisheries Research Station near Lewiston, Michigan, USA (44.86, −84.16). Hunt Creek is a groundwater fed second-order stream in the Thunder Bay River watershed and has never received an annual salmon run (Grossman et al., 2012). Several barriers to upstream movement of fish preclude colonization of Hunt Creek by salmon, which were reasonably abundant in Lake Huron before a major decline in the early to mid-2000s (Cwalinski et al., 2006). A Before-After-Control-Intervention field study design was implemented for this study (Stewart-Oaten et al., 1986). Chinook and Coho ($n = 120$; 50/50 species split) salmon carcasses were introduced into the same salmon "treatment" reach in October 2014 and October 2015 (the typical timing of Michigan salmon runs; Gerig et al., 2018) using loading rates ($\sim$1 kg m^{-2} of stream) approximate to that of a typical salmon run in a Lake Michigan tributary (Janetski et al., 2012; Gerig, 2017). Michigan Department of Natural Resources hatcheries were the source of the salmon carcasses, and salmon died of natural causes. For around 1 year, carcasses were frozen to prevent inadvertent disease introduction to waters that do not have migratory fish runs. Carcasses were then brought to ambient temperature before being staked with rebar in reach habitats, including pools, undercut banks, and debris jams, as has been performed in similar salmon carrion studies (Tiegs et al., 2011). A control reach lacking salmon carcass introduction was located 600 m upstream of the salmon reach (**Figure S1**). Both control and salmon reaches were 90 m long. The average width and depth of the control reach were 0.18 and 3.21 m, and the average width and depth of the treatment reach were 0.19 and 3.61 m, respectively.

Field Sample Collections

Prior to salmon carcass introduction, epinecrotic microbial communities of each carcass were aseptically sampled with sterile and DNA-free cotton swabs using the methods of Pechal and Benbow (2016). Swab samples were individually stored in 200 µL of molecular grade ethanol (>96%) at −20°C. Internal salmon carcass microbial samples were not sampled, so as to not influence the decomposition process by physically altering the salmon carcasses. Microbial and macroinvertebrate communities were sampled at three sub-reaches within the treatment and control reaches: once before (September), once during (October), and four times after (March through August) carcass introduction each year. Sterilized hexagonal unglazed ceramic tiles (29.25 cm^2) were deployed in the stream to characterize epilithic microbial communities (Lang et al., 2016). Six tiles were secured to a brick using a silicone adhesive; five bricks were placed along a transect perpendicular to stream flow in the center of each sub-reach. Bricks were introduced into the stream 2 weeks prior to the first sample collection to establish baseline communities in both reaches. During each collection, the bricks were removed from the stream, a tile was collected and placed in a sterile 188 mL WhirlPak bag (Nasco, Fort Atkinson, WI, USA), kept on ice during transport, and stored at −20°C until DNA extraction. Bricks with the remaining tiles were returned to the same location within the stream. After all tiles were collected, the biofilms were scraped from tiles in the laboratory using autoclaved sterile and decontaminated razor blades into a 2 mL microcentrifuge tube for immediate DNA processing. We did not quantify the amount of microbial growth on any of the collected samples, due to the small amount of growth. During each sampling event, water chemistry parameters of dissolved oxygen (mg/L), pH, conductivity (mS/cm), and temperature (°C) were determined using a YSI 6-Series multiparameter water quality 6600 V2-4 sonde (**Table S1**).

Macroinvertebrates were sampled using a modified Hess sampler (Merritt et al., 2008). At each sub-reach, three riffle habitats were sampled for 30 s each and combined into a single composite for that location (total area = 0.3 m^2). Individual specimens that represented dominant taxa over a variety of feeding groups were hand-picked from the composite Hess sampler collection to ensure adequate sample sizes from representative groups to obtain internal microbial communities; samples were immediately stored in molecular grade ethanol for subsequent internal microbial community analysis. The remainder of the composite Hess sample was stored in 70% ethanol and hand-sorted in the laboratory. Macroinvertebrates were identified to the lowest taxonomic level (genus), except for those used for internal microbiome analyses, which were identified to species (Merritt et al., 2008; Bright, 2016). Functional feeding group was also determined using Merritt et al. (2008) (**Table S2**).Three species were used for internal microbiome analysis due to their abundance and to represent different functional feeding groups: *Heptagenia flavescens* (Walsh) (Ephemeroptera: Heptageniidae; grazer), *Baetis brunneicolor* McDunnough (Ephemeroptera: Baetidae; collector-gatherer), and *Stegopterna mutata* (Malloch) (Diptera: Simuliidae; collector-filterer).

DNA Processing and Targeted 16S rRNA Gene Amplicon Sequencing

For insects, three identified individuals were pooled into one sample and surface sterilized using a 10% bleach rinse followed by three sterile deionized water rinses (Ridley et al., 2012). The insects were air-dried and ground in a 1.7 mL tube using a sterile pestle. DNA extraction was performed with the Qiagen PowerSoil DNA extraction kit® (Qiagen, Inc., Valencia, CA, USA) using a modified manufacturer's protocol: 20 mg mL^{-1} of lysozyme was added during the lysis step and the final DNA was eluted in 50 μL of C6. DNA quantification was performed using the Quanti-iT dsDNA HS Assay kit and a Qubit 2.0 (Grand Island, NY, USA); a concentration of 0.1 ng μL^{-1} was used as a minimum threshold for subsequent sequencing procedures. All DNA preparations were stored at −20°C.

Illumina MiSeq 16S library construction (2 × 250 bp paired-end reads) and sequencing was performed at the MSU Genomics Core using a modified version of the Illumina MiSeq protocol (Caporaso et al., 2011a). The variable region 4 of the 16S rRNA gene was amplified with region-specific primers, 515F/806R (5′-GTGCCAGCMGCCGCGG-3′, 5′-TACNVGGGTATCTAATCC-3′) (Claesson et al., 2010; Caporaso et al., 2011b, 2012). The resulting 16S rRNA amplicon sequencing data were assembled, quality-filtered, and demultiplexed using QIIME2 version 19.1 (Kuczynski et al., 2012). Default settings were used, unless specified in the following methods. DADA2 was used to discard chimeric reads and other sequencing artifacts (Callahan et al., 2016). Taxonomy was assigned using a Naïve Bayes classifier trained using the 16S rRNA region, primer set, read length, and Greengenes 99% reference set version 13.8 (DeSantis et al., 2006; McDonald et al., 2012; Werner et al., 2012), including taxonomy for chloroplasts from eukaryotic microbes. Singletons were removed and samples rarefied to 2,500 sequences, which was the highest sequencing depth that included all biofilm samples (**Figure S2**). Relative abundance was determined by the number of reads in the rarefied dataset. Five samples (four carcass and one internal *H. flavescens*) were excluded due to insufficient sequence reads as a result of extraction or sequencing errors. Sequence files and metadata for all samples used in this study have been deposited in the NCBI SRA under number PRJNA526072.

Carrion-introduced operational taxonomic units (OTUs) in year one were determined by identifying those OTUs detected on salmon carrion prior to deposition in the stream, but not found in samples from either the control reach anytime during year one or the salmon reach before salmon were introduced (September). For year two, carrion-introduced OTUs were those OTUs not detected in year two in the control reach (background OTUs for year two) or during all of year one (both control and salmon reaches and carcasses—background OTUs resulting from any OTUs introduced in year one). The reasoning for excluding year one carcass-associated OTUs from year two carcass introduced, unique OTUs was to evaluate the integration of OTUs that the carrion introduces into biofilms and insects, rather than carrion associated OTUs themselves. Therefore, our strategy was to investigate the microbes completely naïve to the stream biofilms and internal insects during each year's salmon carcass introduction. These targeted sets of unique carrion introduced OTUs (year one and year two) were evaluated for presence in the downstream biofilms and internal insect microbiomes after carcass introduction. In addition, year one unique carrion introduced OTUs were evaluated for presence in year two carrion, to determine what OTUs not found in biofilms or internal insects were introduced both years.

Functional composition of the microbiome was predicted *in silico* using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) on the 16S rRNA amplicon sequences (Langille et al., 2013), using default settings in the online Galaxy version (http://galaxy.morganlangille.com/). PICRUSt analysis requires closed-reference OTU picking using the Greengenes database, thus clustering was conducted on 97% similarity OTUs using VSEARCH in QIIME2 (Rognes et al., 2016). OTUs were normalized by copy number, and predicted functional categories were assigned using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to predict KEGG orthologs, which were then collapsed at level 3 into hierarchical KEGG pathways by function (Kanehisa and Goto, 2000).

Statistical Analyses

Mean ± standard error (MSE) was calculated for each individual taxon. Estimates of α-diversity in microbial communities [observed OTUs, Chao 1 richness, Shannon H', and Faith's phylogenetic diversity (PD) indices] were calculated in QIIME2 based on OTU sequence read matrices (Caporaso et al., 2010). Statistical analyses were performed using R version 1.1.442 (R Core Team, 2018). Estimates of α-diversity in macroinvertebrate communities (genus richness, Simpson's diversity index) were calculated using the vegan 2.5-2 library diversity function in R (Oksanen et al., 2019). Differences in α-diversity metrics for each year's carcass epinecrotic microbial community prior to deposition were tested using unpaired, two-tailed t-tests, while differences in α-diversity metrics in each reach over time were tested separately using repeated measures ANOVA. Residuals vs. fit and normal probability plots were examined to determine if the assumptions of each statistical test were met. When assumptions were not met a Poisson distribution (count data) or $\log_{10}$ transformation (non-count data) was used. Confidence intervals (95%) were determined for coefficient estimates for $\log_{10}$-transformed data. Population densities of the three genera used for internal microbiome testing were examined using the same methods as α-diversity assessments.

Variation in microbial and macroinvertebrate community composition was visualized using non-metric multidimensional scaling (NMDS) and statistically evaluated for β-diversity metrics with PERMANOVA, a phylogeny based matrix (weighted UniFrac) for microbial communities and Bray-Curtis dissimilarity matrix for macroinvertebrate communities (standard for macroinvertebrate community analysis) using 99,999 permutations in the vegan 2.5-2 library "adonis" function in R (Anderson, 2001). For those samples where stream reach was statistically significant, we identified taxa (genus level for macroinvertebrates and family level for microbes) found in the salmon reach over time via indicator species analysis (ISA) with Indicator Value (IndVal) Index and its significance using 99,999 permutations in the "signassoc" function in the R package indicspecies, with p-value adjustments for multiple comparisons using the Sidak method (Dufrêne and Legendre, 1997; Cáceres and Legendre, 2009; Cáceres et al., 2010). All other statistical tests were considered significant at $\alpha = 0.05$. Due to statistically significant differences in salmon carrion conditions during each year of the study (see section Results, **Tables S3–S5**), separate analyses were conducted for each year for all response variables.

RESULTS

Macroinvertebrate Community Composition

A total of 13,730 aquatic macroinvertebrates were identified comprising 49 taxa, with Chironomidae the most relative abundant and a mean of 17% (±2%) (**Table S6**). No covariate (salmon treatment, time, or their interaction) was found to significantly influence macroinvertebrate density or richness in either year of the study ($p > 0.05$). Although no covariate influenced diversity during year one, in year two macroinvertebrate diversity increased by 0.0015 (± 0.0005) each day ($p < 0.01$), and there was a significant time × treatment interaction ($p = 0.04$). Only time had significant effects on macroinvertebrate community structure during both years (PERMANOVA: $p < 0.01$, **Table 1**). In year two, *Brachycentrus* (collector-filterer) density (individuals per 0.3 m^2) was lower in the salmon reach relative to the control reach [$\bar{x}_S = 0$ (±0), $\bar{x}_C =$ 6 (±3), ISA: $p = 0.03$, **Figure S3**]. *Heptagenia* population density significantly increased in year one and was also significantly higher in the salmon reach during both years [$\bar{x}_S = 14$ (±4), $\bar{x}_C = 3$ (±1), ANOVA: $p < 0.02$, **Figure S3**]. *Baetis* density significantly increased each year, with a significant time × treatment interaction due to a higher abundance in the control reach 9 months after salmon introduction during both years [$\bar{x}_S = 41$ (±14), $\bar{x}_C = 191$ (±41), $p < 0.01$, **Figure S3**]. We found a significant time × treatment interaction influencing *Stegopterna* density in year one, due to decreased abundance in the salmon reach 9–11 months after salmon introduction [$\bar{x}_S = 19$ (±6), $\bar{x}_C = 109$ (±44), $p < 0.01$]. *Stegopterna* density also significantly increased over time during year two ($p < 0.01$).

TABLE 1 | PERMANOVA results testing benthic macroinvertebrate community structure based on Bray-Curtis distances for each year of the study with significant results ($p < 0.05$) indicated by an asterisk.

Factor	df	SS	MS	F	R^2	P
YEAR ONE						
Treatment	1	0.35	0.35	1.63	0.03	0.11
Time	1	1.93	1.93	9.02	0.18	<0.01*
Treatment × Time	1	0.23	0.23	1.07	0.02	0.34
Residuals	38	8.15	0.21		0.76	
Total	41	10.66			1.00	
YEAR TWO						
Treatment	1	0.39	0.39	1.63	0.05	0.11
Time	1	0.86	0.86	3.56	0.10	<0.01*
Treatment × Time	1	0.22	0.22	0.90	0.02	0.50
Residuals	32	6.99	0.24		0.83	
Total	35	8.46			1.00	

df, degrees of freedom; SS, sum of squares; MS, mean sum of squares.

Salmon Carcass Epinecrotic Community Composition

A total of 11,219 microbial OTUs representing 51 phyla were identified in the carcass microbial communities. *Moraxellaceae* (γ-Proteobacteria) had the highest relative abundance [16% (±4%)]. While the diversity metrics Faith's PD and Chao 1 were not significantly different for each year of introduction (t-test: $p > 0.1$), the epinecrotic microbial communities were different between years, both taxonomically (OTU level) and functionally (PERMANOVA: $p < 0.01$, **Table S3**). Twenty microbial families were indicators of the year in the epinecrotic communities (ISA: $p < 0.05$, **Table S4**). *Ruminococcaceae*, *Geobaceracae*, *Succinivibrionaceae*, *Spirochaetaceae*, an unknown family in *Bacteroidales*, and an unknown family in *YS2* were the most significant indicator families ($p<0.01$) and were all only found in year one carcasses. The most abundant indicator family, *Sphingomonadaceae*, had 2.7 times higher relative abundance in year one carcasses than year two carcasses [$\bar{x}_{Y1} = 17\%$ (±3%), $\bar{x}_{Y2} = 6\%$ (±1%)]. Functionally, 135 KEGG orthologs were indicators of salmon carcass introduction year (ISA: $p < 0.05$, **Table S5**). The most significant KEGG orthologs were caffeine metabolism, ether lipid metabolism, ethyl benzene degradation, isoflavonoid biosynthesis, mineral absorption and proteasome, all of which were greatest in year two carcass microbial communities ($p < 0.01$). The most abundant salmon indicator KEGG ortholog was DNA repair and recombination protein, which was 1.4 times higher in year two carcasses [$\bar{x}_{Y1} = 41{,}587$ (±2,865), $\bar{x}_{Y2} = 57{,}353$ (±3,244), ISA: $p = 0.02$]. Melanogenesis, a salmon indicator KEGG ortholog, was 3 times higher in year two [$\bar{x}_{Y1} = 8$ (±2), $\bar{x}_{Y2} = 25$ (±4), ISA: $p < 0.01$].

Biofilm Community Composition

A total of 11,051 and 9,434 OTUs represented epilithic biofilm communities in year one and year two, respectively, from 72 total samples (36 per year). The most abundant family was an unnamed family in the order Stramenopiles, representing 17% (±2%) of the community. Faith's PD significantly decreased over time during both years (year one: 2–3%, year two: 1–2%, ANOVA: $p < 0.01$), while Chao 1 richness increased over year one (0.6–0.8%, $p < 0.01$) and decreased in year two (0.4–0.6%, $p < 0.01$), but salmon treatment did not have an effect on Faith's PD or Chao 1 richness ($p > 0.05$). Treatment, time, and a treatment × time interaction influenced community composition during year one (PERMANOVA, $p < 0.05$, **Table 2A**, **Figure S4**), but only time was significant in year two.

Fifteen families were significant representatives of salmon reach biofilm communities in year one ($p < 0.05$, **Table S7**). *Alteromonadaceae* was 3.5 times more abundant in the salmon reach [ISA: $p < 0.01$, **Figure 2**]. Only two indicator families represented >10% of the community composition: *Saprospiraceae* and the abovementioned unnamed family in the order Stramenopiles. *Saprospiraceae* was 17 times lower in abundance in the salmon reach [0.7% (±0.2%)] compared to the control reach [12% (±3%)] 2 weeks after salmon introduction in year one, while an unnamed family in the order Stramenopiles exhibited the same pattern during both years, having 30 and 2.5 times lower abundance in the salmon reach in year one [$\bar{x}_S = 2\%$ (±2%), $\bar{x}_C = 61\%$ (±9%)] and year two [$\bar{x}_S = 25\%$ (±13%), $\bar{x}_C = 60\%$ (±4%)], respectively. Three indicator families identified from biofilms after carcass introduction were also indicator families of the salmon carcass microbial communities: *Sphingomonadaceae* [$\bar{x}_S = 1.8\%$ (±0.4%), $\bar{x}_C = 0.9\%$ (±0.1%)], *Geobacteraceae* [$\bar{x}_S = 0.12\%$ (±0.04%), $\bar{x}_C = 0.04\%$ (±0.02%)], and *Xanthomonadaceae* [$\bar{x}_S = 1.0\%$ (±0.2%), $\bar{x}_C = 0.6\%$ (±0.1%)], all of which had higher mean relative abundance in the salmon reach.

In year one, treatment and time (but not interaction) significantly influenced the composition of KEGG orthologs (PERMANOVA, $p < 0.05$, **Table 2A**, **Figure S4**), yet there were no significant effects in year two. In year one biofilms, 113 indicator KEGG orthologs ($p < 0.05$, **Table S8**) were identified, with the most significant carcass KEGG ortholog indicator being fluorobenzoate degradation [$\bar{x}_S = 1{,}760$ (±36), $\bar{x}_C = 1{,}580$ (±42), ISA: $p < 0.01$], and the most abundant was the two-component system [$\bar{x}_S = 61{,}151$ (±148), $\bar{x}_C = 55{,}800$ (±154), ISA: $p = 0.04$], both of which were higher in the control reach. A total of 41 KEGG orthologs indicated salmon treatment biofilm communities, as well as year of carcass introduction. Of those shared indicator KEGG orthologs with higher abundance in the salmon reach, phosphotransferase system was the most abundant [$\bar{x}_S = 1{,}805$ (±106), $\bar{x}_C = 1{,}411$ (±163), ISA: $p < 0.01$]. Another one of those shared KEGG orthologs was melanogenesis, which was 1.7 times higher in abundance in salmon reach biofilms [$\bar{x}_S = 17$ (±3), $\bar{x}_C = 10$ (±2), ISA: $p < 0.01$].

Aquatic Insect Internal Microbial Community Composition

In the mayfly *B. brunneicolor*, 1,898 and 2,269 OTUs were detected in year one and year two, respectively (47 total samples with 3 individuals each). In year one, an unnamed family in *Mollicutes* was the most relatively abundant family [13% (±4%)], while in year two the most abundant was *Pseudomonadaceae* [23% (±4%)]. Neither time nor treatment significantly influenced Chao 1 richness (ANOVA: $p > 0.05$), yet Faith's PD decreased over time in year one (2.2–9.3%, $p < 0.01$) and year two (0.4–1.7%, $p < 0.01$). In year two, we also observed 99% lower Faith's PD in the salmon reach (31–100%), and a significant time × treatment interaction ($p < 0.05$).

Six families were indicators of internal microbial communities of *B. brunneicolor* in the salmon reach ($p < 0.05$, **Table S9**). Of these six, the most significant and abundant was the aforementioned unnamed family in *Mollicutes*, which was five times greater in the control reach [$\bar{x}_S = 5\%$ (±2%), $\bar{x}_C = 25\%$ (±7%), ISA: $p < 0.01$]. An unknown family in *Rhizobiales* (α-Proteobacteria) was an indicator of microbial communities in both internal *B. brunneicolor* from the salmon reach and from salmon carcass communities of year one, with 3.5 times higher abundance in the salmon reach [$\bar{x}_S = 7\%$ (±2%), $\bar{x}_C = 2\%$ (±1%), ISA: $p < 0.01$]. Only time significantly influenced the microbial community composition of the *B. brunneicolor* internal microbiome during year two (PERMANOVA: $p = 0.02$, **Table 2B**), while neither time nor treatment had significant effects in year one or influenced the KEGG orthologs detected in biofilms in either year ($p > 0.05$).

In the black fly *S. mutata*, a total of 449 and 1224 OTUs were detected in year one and year two, respectively (23 total samples with 3 individuals each), with Firmicutes being the predominant phylum [32% (±5%)]. Although no significant factors influenced Faith's PD in year one, in year two, mean diversity was 72 (±33) times higher in internal *S. mutata* in the salmon reach compared to the control reach (ANOVA: $p = 0.05$). Chao 1 richness was not influenced by time or treatment during either year ($p > 0.05$).

Treatment, time, nor their interaction significantly affected the microbial composition or functional KEGG ortholog community composition of internal *S. mutata* in year one (PERMANOVA: $p > 0.05$). In year two, the treatment × time interaction significantly influenced both the internal microbial community structure and function ($p < 0.02$, **Table 2C**). An unnamed family in Streptophyta was an indicator family of treatment in year two, with four times greater relative abundance in the control reach internal *S. mutata* [$\bar{x}_S = 1.1\%$ (±0.4%), $\bar{x}_C = 4\%$ (±1%), ISA: $p < 0.03$]. Melanogenesis was the only indicator KEGG ortholog for *S. mutata* internal communities from the salmon reach in year two, with four times greater relative abundance in the control reach [$\bar{x}_S = 21$ (±4), $\bar{x}_C = 61$ (±16), ISA: $p = 0.05$], which was also an indicator KEGG ortholog in salmon carcasses and biofilms (**Figure S5**).

TABLE 2 | PERMANOVA results testing microbial community structure based on the weighted phylogenetic distance (UniFrac) matrix for β-diversity and Jaccard distance matrix for KEGG orthologs among the microbial communities from the 2 years' biofilms **(A)**, internal *B. brunneicolor* **(B)**, and internal *S. mutata* **(C)**, with significant results ($p < 0.05$) indicated by an asterisk.

		OTUs					KEGG orthologs				
Factor	**df**	**SS**	**MS**	***F***	***R*²**	***P***	**SS**	**MS**	***F***	***R*²**	***P***
(A) Biofilm											
Year one											
Treatment	1	0.12	0.12	2.58	0.05	0.05*	0.04	0.04	2.87	0.07	0.05*
Time	1	0.69	0.69	14.75	0.27	<0.01*	0.08	0.08	5.16	0.12	<0.01*
Treatment × Time	1	0.22	0.22	4.66	0.09	0.01*	0.03	0.03	2.29	0.05	0.09
Residuals	32	1.50	0.05		0.59		0.48	0.02		0.76	
Total	35	2.53			1.00		0.64			1.00	
Year two											
Treatment	1	0.12	0.12	1.59	0.04	0.15	0.03	0.03	1.07	0.03	0.32
Time	1	0.53	0.53	7.37	0.17	<0.01*	0.02	0.02	0.89	0.02	0.40
Treatment × Time	1	0.12	0.12	1.69	0.04	0.14	0.02	0.02	0.91	0.03	0.39
Residuals	32	2.32	0.07		0.75		0.82	0.03		0.92	
Total	35	3.09			1.00		0.89			1.00	
(B) *B. brunneicolor*											
Year one											
Treatment	1	0.06	0.06	0.76	0.05	0.71	0.01	0.01	0.09	0.01	1.00
Time	1	0.17	0.17	2.16	0.13	0.07	0.03	0.03	0.30	0.02	0.80
Treatment × Time	1	0.05	0.05	0.59	0.03	0.81	0.02	0.02	0.16	0.01	0.95
Residuals	13	1.04	0.08		0.79		1.24	0.10		0.96	
Total	16	1.32			1.00		1.29			1.00	
Year two											
Treatment	1	0.15	0.15	1.23	0.04	0.32	0.10	0.10	1.02	0.04	0.33
Time	1	0.44	0.44	3.54	0.11	0.02*	0.08	0.08	0.88	0.03	0.39
Treatment × Time	1	0.12	0.12	0.98	0.03	0.35	0.10	0.10	1.01	0.03	0.34
Residuals	26	3.21	0.12		0.82		2.45	0.09		0.90	
Total	29	3.92			1.00		2.72			1.00	
(C) *S. mutata*											
Year one											
Treatment	1	1.92	1.92	5.69	0.58	0.09	0.03	0.03	0.84	0.08	0.49
Time	1	0.09	0.09	0.26	0.03	0.70	0.13	0.13	3.30	0.32	0.13
Treatment × Time	1	0.27	0.27	0.81	0.08	0.42	0.13	0.13	3.17	0.31	0.10
Residuals	3	1.01	0.34		0.31		0.12	0.04		0.29	
Total	6	3.29			1.00		0.41			1.00	
Year two											
Treatment	1	0.10	0.10	1.36	0.06	0.15	0.02	0.02	0.19	0.01	0.89
Time	1	0.15	0.15	2.16	0.10	0.10	0.06	0.06	0.51	0.03	0.57
Treatment × Time	1	0.27	0.27	3.89	0.18	<0.01*	0.69	0.69	6.39	0.30	0.01*
Residuals	14	0.97	0.07		0.66		1.50	0.11		0.66	
Total	17	1.49			1.00		2.27			1.00	

df, degrees of freedom; SS, sum of squares; MS, mean sum of squares.

The internal microbiome of the mayfly *H. flavescens* could not be compared between treatment and control reaches due to low-yield microbial DNA and a low sample size ($n = 8$).

Introduced Salmon Carcass Microbes

Of the total 686 salmon carcass-derived OTUs introduced in year one, 645, representing an average relative abundance of 63 ± 3%, were not found in biofilm or internal insect samples in non-salmon reaches (unique) (**Figure S6**). During year two, 1,786 [51% (±6%)] of a total of 2,196 were OTUs associated with introduced carcasses and not found in biofilm or internal insect samples anytime during year one, or in non-salmon reaches. Of unique OTUs introduced via carrion in the first year, 31 were detected in treatment biofilms (**Table S10**), of which 21 were only found in year one biofilms, six were only found in year two biofilms, and four were found in both years (**Figure S6**). Of the unique OTUs introduced via carrion in year two, 25 were detected in year two treatment biofilms. However, all unique OTUs introduced via salmon carrion and found in biofilms represented <2% of biofilm communities, except for a pulse 2

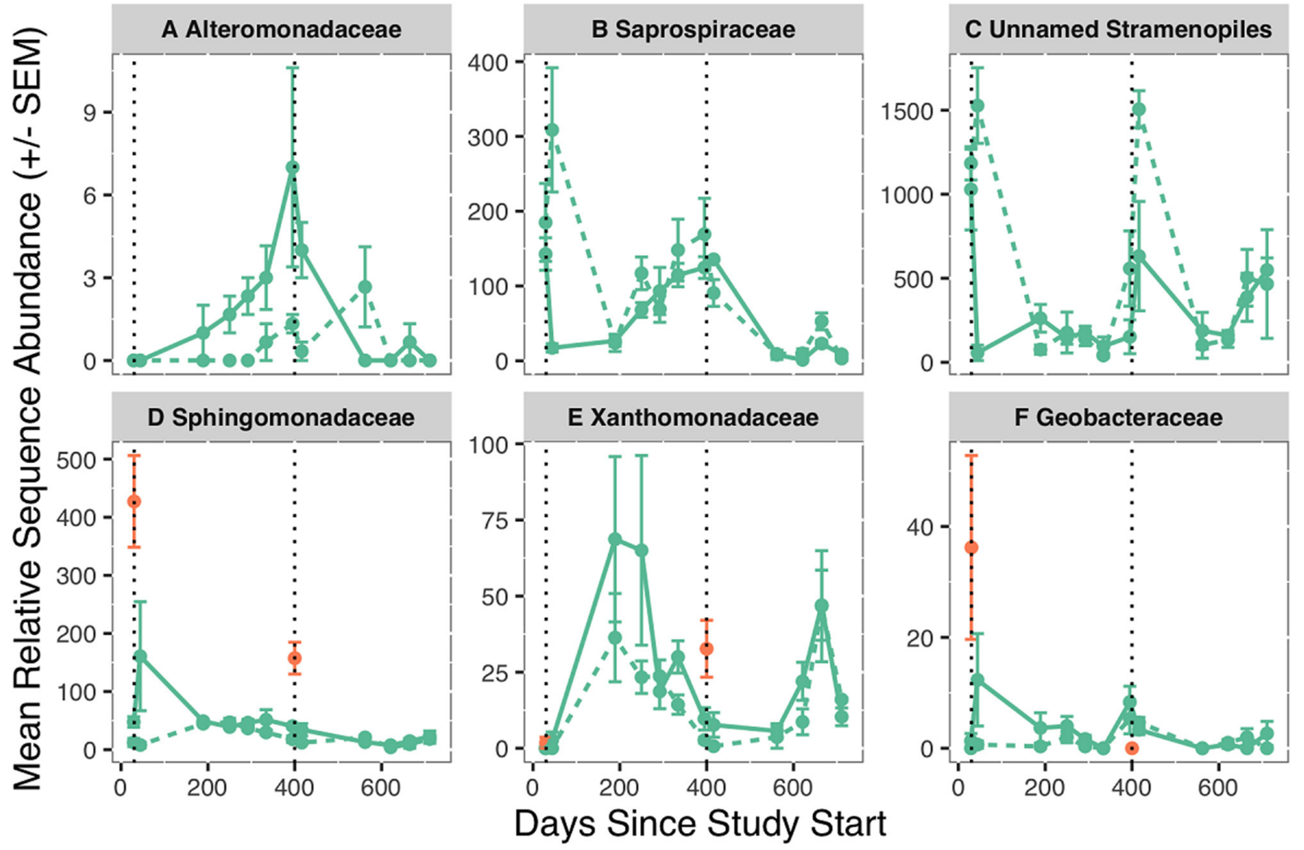

FIGURE 2 | Mean relative sequence abundance (±SEM) of *Alteromonadaceae* **(A)**, *Saprospiraceae* **(B)**, Unnamed Stramenopiles family **(C)**, *Sphingomonadaceae* **(D)**, *Xanthomonadaceae* **(E)**, and *Geobacteraceae* **(F)** in biofilms (green) over time. *Sphingomonadaceae*, *Xanthomonadaceae*, and *Geobacteraceae* that were part of the carrion associated communities are shown in orange, as these families were also indicator taxa for year of salmon carrion addition within the salmon carrion microbial communities. The dashed line is the control reach, and the solid line is the salmon reach. Black, dotted vertical lines represent the day of salmon introduction. Relative sequence abundance is the number of reads in the rarefied (2,500 reads) dataset.

weeks after carcass introduction in year one when they increased to 5% (±1%) (**Figures 3A** and **3D**). Three year one, salmon carrion unique OTUs found in biofilms were found in the upstream control reach in year two.

Of the OTUs introduced via carrion in year one, nine were found in the internal *B. brunneicolor* communities collected in the salmon reach: three, five, and one OTU(s) were detected in year one, year two, and both years, respectively (**Figure S7**, **Table S10**). Four of these OTUs were also detected in treatment biofilms (**Figures 3A** and **3B**). Of those unique OTUs introduced via carrion in year two, eight persisted in year two treatment *B. brunneicolor* internal communities, none of which were found in treatment biofilms or *S. mutata*. However, these carrion-introduced unique OTUs represented <1% of the relative abundance of internal *B. brunneicolor* communities (**Figures 3B** and **3E**); three year one introduced OTUs found in internal *B. brunneicolor* were also found in the upstream control reach in year two.

Three OTUs introduced by carrion in year one and not found in biofilm or internal insect samples in non-salmon reaches were detected in internal *S. mutata* collected in the salmon reach: one in year one and two in year two (**Figure S7**, **Table S10**). None of these OTUs were found in biofilms or *B. brunneicolor*. Of the unique salmon carrion OTUs introduced in year two, 14 were detected in year two treatment internal communities of *S. mutata*. Three of those fourteen were also detected in treatment biofilms in year two. These introduced OTUs from both years represented a small proportion (<1%) of internal *S. mutata* communities (**Figures 3C** and **3F**). In year two, four year one introduced OTUs found in internal *S. mutata* were also found in the upstream control reach.

DISCUSSION

Macroinvertebrate Community Composition

Specific metrics of α-diversity and population density of macroinvertebrate communities were altered by salmon carrion additions, which were influenced by the population dynamics of four taxa: *Brachycentrus*, *Baetis*, *Stegopterna*, and *Heptagenia*. Higher Brachycentridae abundance has been detected 2–4 weeks after salmon carcass introduction in Idaho (Kohler et al., 2008),

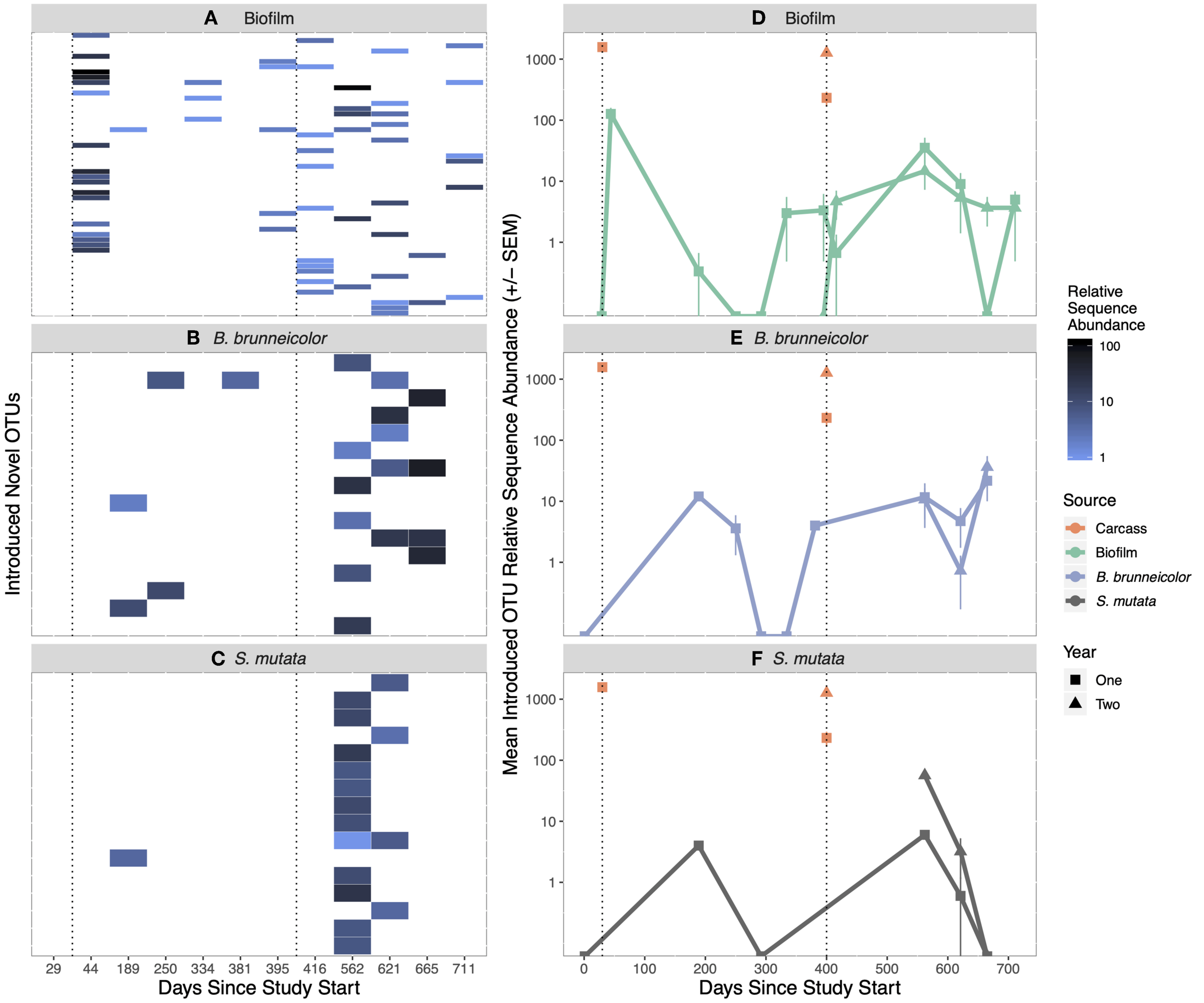

FIGURE 3 | Heatmap of unique salmon carrion introduced OTUs found in treatment biofilm **(A)**, internal *B. brunneicolor* **(C)**, and internal *S. mutata* **(E)** after carcass introduction. Each y-axis row on the heatmap represents one unique salmon carrion introduced OTU. White color in heatmaps represents zero observations. Mean introduced OTU abundance (±SEM) in salmon carcasses and biofilms **(B)**, internal *B. brunneicolor* **(D)**, and internal *S. mutata* **(F)**, over time. The orange carcass points represent the unique OTUs introduced to the stream for that year. The carcasses added in year 2 contained OTU's previously introduced in year 1 (orange square), as well as new OTU's not previously found in biofilm or insect samples (orange triangle). The sample (biofilm, internal *B. brunneicolor*, or internal *S. mutata*) points (in green, purple or gray, respectively) represent the amount of that year's salmon introduced OTUs found in biofilm samples. Black, dotted vertical lines represent the day of salmon introduction. Relative sequence abundance is the number of reads in the rarefied (2,500 reads) dataset.

and *Brachycentrus* has been observed feeding on salmon carcasses in Alaska (Kline et al., 1997). Yet, in our study, *Brachycentrus* density was lower in the salmon reach, never representing more than 1% of the community, which did not support the hypothesis that collectors would increase in the salmon treatment reach. In Michigan, *Brachycentrus* populations can be drastically reduced by a microsporidium parasite (Kohler and Hoiland, 2001), which has an unknown life cycle. It is possible that salmon carrion introduces microsporidium spores, and a local outbreak could have lowered *Brachycentrus* population in the salmon reach, although there is no direct historical evidence of this parasite in Hunt Creek (Wills et al., 2006), and the presence of this parasite or its spores were not directly measured in this study. Additional salmon carcass introduction studies in other Great Lakes streams with *Brachycentrus* are needed to determine whether these observed changes were due to natural environmental shifts that occurred upstream of the salmon reach or a treatment effect. Changes in the phenology of this insect may be more important in structuring the population than the availability of resources. Great Lakes region salmon research shows that salmon carrion has a much smaller impact on stream biota than it does in its native range in the Pacific Northwest and Alaska (Janetski et al., 2013), leaving phenological population changes to have a greater impact.

Although the *Brachycentrus* population was consistently higher in the control reach, *Baetis* and *Stegopterna* populations were higher only during a short time peak in the control reach compared to the salmon reach. Both taxa belong to the collector functional feeding group, and were found to increase in density or have no significant response to salmon carrion subsidies in Alaska (Wipfli et al., 1998, 1999; Minakawa and Gara, 1999; Chaloner et al., 2002, 2004; Lessard et al., 2009). In the few studies that show lower collector densities in salmon-bearing streams, this was attributed to benthic disturbance by live salmon spawning behavior (Honea and Gara, 2009; Collins et al., 2011), which was not a factor in this study, as we introduced salmon carcasses directly to a naïve stream. Earlier insect emergence in streams that experience annual salmon runs could be attributed to an insect evolutionary response to salmon spawning disturbance (Moore and Schindler, 2010). Alternatively, the salmon nutrient subsidy may also lead to earlier emergence because of increased production and faster growth rate in insects. The short 50 years of evolutionary history of salmon in Great Lakes streams may preclude such responses in taxa such as *Baetis* and *Stegopterna*.

Although *Brachycentridae, Baetis*, and *Stegopterna* populations had higher mean relative abundance in the control reach*,* *Heptagenia* were higher in the salmon reach. Mayfly grazers, such as heptageniids, have been found to consume periphyton containing salmon-derived nitrogen (Schuldt and Hershey, 1995). Therefore, a salmon nutrient subsidy may have had positive effects on the *Heptagenia* population, supporting the hypothesis that grazer macroinvertebrates would increase in abundance in the salmon treatment reach.

Microbial Community Structure

The microbial communities residing in benthic biofilms were altered by carcass introduction over time for both years of the study, but this impact differed in each year. The introduced carcasses supported different microbial communities between the 2 years, which may contribute to this variation. Specifically, melanogenesis, a pathway responsible for pigment production, was an indicator KEGG ortholog in salmon carrion microbial communities, as well as in biofilms and internal *S. mutata*, but with contrasting effects. Melanin pigment in microbes is associated with virulence in pathogens and protection against environmental stressors (Nosanchuk and Casadevall, 2003). Each year's salmon carcasses were raised in different environments, causing more melanogenesis in year two salmon epinecrotic microbial communities. Then, the melanogenesis performing microbes became integrated into salmon treatment biofilms in year two, so there was increased melanogenesis in salmon treatment biofilms compared to control sites. This functional pathway existed in the stream prior to salmon carrion introduction, but salmon could have enriched the OTUs already present in biofilms, leading to higher abundance in that treatment reach. In contrast, the internal microbial communities within *S. mutata* had elevated melanogenesis in the control reach. This elevation may be due to an environmental change in the treatment reach due to salmon introduction, such as increased dissolved organic carbon (Schuldt and Hershey, 1995; Collins et al., 2011), which may decrease the abundance of microbes that perform melanogenesis. It should also be noted that KEGG orthologs are predicted via *in silico* analysis of the microbial community datasets, and further studies directly measuring microbial functions are needed.

Another shift in biofilm composition involved an unnamed family in Stramenopiles in year one, which was lower in the salmon reach compared to the control reach 2 weeks after carcass introduction. Functionally, Stramenopiles are a dominant group of primary producers (Burliga and Kociolek, 2016). We would expect Stramenopiles to be more abundant after leaf fall, due to increases in light with less canopy cover (Sumner and Fisher, 1979). Leaf fall occurred at the same time that salmon carrion was introduced into the stream. Primary production only marginally increases due to nutrients released by salmon carrion in Great Lakes streams (Schuldt and Hershey, 1995; Hershey and Wold, 1999); however, this production can be altered by stream conditions, such as light availability, habitat structure, and organic material (Cederholm et al., 1999). Nutrient addition in a Tennessee stream increased primary production in the autumn with increased light availability, but this effect was significantly lower in the presence of grazers (Rosemond et al., 2000). We detected an increase in grazer *Heptagenia* in the salmon reach, and thus these grazer communities could have influenced the biofilm response and limited autotrophic microbes despite nutrient inputs from salmon carrion.

In contrast to Stramenopiles, the *Sphingomonadaceae* (α-Proteobacteria) were over twice as abundant in the salmon reach, suggesting that heterotrophic bacteria respond positively to salmon carrion subsidies. Some of this increase in heterotrophic microbes can be attributed to rare OTUs introduced via salmon carrion, but most are likely due to an increase in organic matter. Benthic biofilms in streams with higher dissolved organic carbon often have higher Proteobacteria relative abundance (Gao et al., 2005). Proteobacteria also was found to be in high relative abundance in the internal microbiome of a predator mayfly in salmon-bearing streams (Pechal and Benbow, 2016); we found a similar trend with an unknown family in *Rhizobiales* (α-Proteobacteria), which had 3.5 times higher abundance in the internal microbiome of *B. brunneicolor* in the salmon reach of Hunt Creek.

Temporal Dynamics

Early research into salmon carcass decomposition in streams have indicated that periphyton first use salmon nutrients followed by primary consumers (Juday et al., 1932; Mathisen et al., 1988). Salmon-derived nutrients were found to peak in insects directly feeding on carcasses at 2 weeks after introduction, while biofilms and insects that were indirectly affected by salmon carrion had a peak in salmon derived nutrients at 2 months after introduction (Claeson et al., 2006). We found that biofilms responded 2 weeks after introduction, integrating a small amount (<2%) of unique salmon introduced OTUs into these epilithic communities, suggesting a more direct uptake path. Additionally, we were only able to sample a small amount of the total introduced microbial diversity via salmon in our carcass surface swabs, because we were not able to monitor the introduction of microbes from the gastrointestinal (GI) tract. We speculate the GI microbes may also contribute to novel taxa found in biofilms and internal insects that were not detected in this small relative abundance. The macroinvertebrate communities shifted several months after introduction, suggesting indirect and lagged carcass resource use. In our study, the small number of microbes unique to carrion and integrated into biofilms are subsequently integrated into consumers, as nutrients would be integrated up the food chain. An alternative explanation is that naïve Hunt Creek does not contain the necrophilous invertebrates of a typical Pacific salmon stream, and future research should focus on the direct and indirect pathways of introduced microbes.

It should be noted that the number of reads in a sequencing dataset do not necessarily directly translate to abundance in the environment, but rather serves as a proxy. Additionally, the detection of OTUs in biofilms does not indicate living microbes, but that the DNA of those microbes was present. Residual DNA from the salmon may slough off and be retained in biofilms, without the bacteria reproducing and functioning in the environment. Further studies, using active, transcribed forms of DNA are necessary to mechanistically determine whether these unique OTUs play an environmentally significant role. Past studies have shown that rare microbial taxa may play vital roles in maintaining biodiversity and having functional roles (Shade et al., 2014; Lynch and Neufeld, 2015; Jousset et al., 2017). For example, rare taxa can provide a “seed bank” that may increase in abundance when there is a local extinction of more abundant taxa or immigrate to another habitat where it can outcompete other resident microorganisms. Therefore, despite the low abundances of carrion-introduced OTUs in our system, they may play a more disproportionate role in biodiversity and ecosystem functioning that future studies should investigate.

CONCLUSIONS

In this study, we contribute to knowledge on ecology of salmon carrion decomposition by investigating the microbial fauna of a naïve stream following a salmon carcass addition. These data provide evidence that salmon introduce microbial taxa to recipient streams, and a small amount become incorporated into the ecosystem. Further, these taxa may elicit a cascading effect that influences stream producer and consumer communities through direct and indirect pathways. Salmon migration may ignite complex interkingdom interactions in stream communities, necessitating additional field and laboratory studies on allochthonous sources of microbes and their potential importance and mechanisms to ecosystem function. Therefore, the functional roles of these salmon-associated microbial taxa represent a frontier for ecological research.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the NCBI SRA under number PRJNA526072 [https://www.ncbi.nlm.nih.gov/bioproject/PRJNA526072].

AUTHOR CONTRIBUTIONS

CL designed the microbial studies, collected the macroinvertebrate and microbial data in the field, analyzed the macroinvertebrate and microbial data, and wrote the paper. JP contributed to the study design, assisted in the field, and edited the paper. BG designed the overall experiment, assisted in the field, and edited the paper. DC assisted with experimental design and edited the paper. GL contributed to experimental design and edited the paper. MB contributed to study design, assisted in the field, and contributed to writing and editing the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00505/full#supplementary-material

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Landscape Structure and Species Interactions Drive the Distribution of Salmon Carcasses in Coastal Watersheds

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The disproportionate effects of some species can drive ecosystem processes and shape communities. This study investigates how distributions of spawning Pacific salmon within streams, salmon consumers, and the surrounding landscape mediate the distribution of salmon carcasses to riparian forests and estuaries. This work demonstrates how carcass transfer can vary spatially, within and among watersheds, through differences in pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon distributions within 16 streams on the central coast of British Columbia over a five-year period. Spawning pink salmon concentrated in the lower reaches of all streams, whereas chum salmon shifted from lower to upper stream reaches as the area of spawning habitat increased. Salmon carcasses transferred to riparian areas by gray wolves (*Canis lupus*) were concentrated in estuaries and lower stream reaches, particularly shallow reaches of larger streams surrounded by large meadow expanses. Black and grizzly bears (*Ursus americanus* and *U. arctos*) transferred higher numbers and proportions of salmon carcasses to riparian areas compared to wolves, transferred more carcasses in areas of higher spawning density, and tended to focus more on chum salmon. Riparian subsides were increasingly driven by bear-chum salmon associations in upper stream reaches. In addition, lower proportions of salmon carcasses were exported into estuaries when densities of spawning salmon were lower and spawning reaches of streams were longer. This study demonstrates how salmon subsidies vary between and within watersheds as a result of species associations and landscape traits, and provides a nuanced species-specific and spatially explicit understanding of salmon-subsidy dynamics.

Keywords: cross-ecosystem, fisheries, landscape ecology, nutrient subsidies, pacific salmon

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INTRODUCTION

Cross-boundary resource exchanges in material, organisms and energy can form a major component of resource bases within ecosystems (Polis et al., 1997; Anderson et al., 2008). Some linkages can be driven by large-scale processes such as El Niño affecting ecosystems from the Galápagos Islands to Australia (Holmgren et al., 2001), or trans-oceanic winds bringing iron from

African deserts to South American forests (Bristow et al., 2010). Other nutrient linkages can have more localized effects, such as reciprocal flows of invertebrates linking terrestrial and freshwater food webs (Power, 2001; Baxter et al., 2005). In some cases, landscape structure can play a key role in mediating the delivery of resources across ecosystems (Turner, 1989; Polis et al., 1997; Loreau and Holt, 2004).

Pacific salmon (*Oncorhynchus* spp.) provide one of the most ecologically important examples of broad-scale, cross-boundary life histories in the animal kingdom (Groot and Margolis, 1991; Janetski et al., 2009). Freshwater habitats mark the beginning and end of a life cycle mostly spent in productive oceanic feeding grounds. Semelparous salmon rear in and then return to natal streams as adults to reproduce, thereby importing mass quantities of marine-derived material into coastal ecosystems throughout the North Pacific Rim. A wide body of research has documented the importance of Pacific salmon in linking offshore marine productivity to coastal ecosystems (Cederholm et al., 1999; Gende et al., 2002; Naiman et al., 2002). After spawning, their nutrient-rich carcasses are dispersed along streams, estuaries, and into adjacent forests by consumers and the movement of water Cederholm et al., 1989; Payne and Moore, 2006; Quinn et al., 2009.

Black and grizzly bears (*Ursus americanus* and *U. arctos*) can transfer large quantities of salmon-derived material from streams to riparian forests (Frame, 1974; Hilderbrand et al., 1999; Reimchen, 2000, 2017). Bears exploit this predictable and accessible annual pulse of protein, which constitutes a crucial resource during their preparation for winter dormancy (Quinn et al., 2003; Hilderbrand et al., 2011). Gray wolves (*Canis lupus*) are another major consumer that depend on salmon and can transfer significant numbers of salmon carcasses to riparian areas (Darimont et al., 2003). Isotope evidence suggests that coastal wolves shift their diet from ungulates to salmon during fall spawning events (Darimont and Reimchen, 2002; Darimont et al., 2008). Salmon carcasses deposited in riparian forests by these consumers can increase soil organic content (Bartz and Naiman, 2005; Gende et al., 2007), elevate nutrient concentration, shift the diversity of riparian plant communities (Bilby et al., 2003; Hocking and Reynolds, 2011), and provide substantial resources to terrestrial invertebrate communities (Hocking et al., 2009, 2013). Although salmon play important roles in stream ecosystems as a source of nutrients and disturbance to stream beds through the action of digging redds (Janetski et al., 2009; Tiegs et al., 2009; Harding et al., 2014), considerable proportions of carcasses are also exported to estuaries (Gende et al., 2004b), which can elevate dissolved nutrient concentrations and provide substantial resource inputs into estuarine food webs (Cak et al., 2008; Harding and Reynolds, 2014a; Harding et al., 2015). However, differences in salmon nutrient input to estuaries likely vary as a function of spawner distributions and stream size as carcasses are usually transported limited distances downstream and are often retained within pools and organic debris within streams (Cederholm and Peterson, 1985; Minakawa and Gara, 2005; Strobel et al., 2009).

To date, Pacific salmon species have generally been grouped together by their effects as resource subsidies and sources of streambed disturbance despite inter-species variation in life histories (c.f. Service et al., 2018). While all Pacific salmon have the potential to subsidize coastal ecosystems to some extent, contrasts in how each species responds to different habitats may contribute to more complex relationships between carcass dispersal mechanisms and thus the subsidy potential of each salmon species (Hooper et al., 2005). A crucial consideration when assessing the subsidy potential of salmon is their distribution within streams. Variation in the distribution of live spawning salmon between species, or amongst streams, will influence the distribution of salmon-derived nutrients. The magnitude of salmon carcass transfer to riparian forests by consumers and the export of carcasses downstream may also vary spatially based on variability in spawning salmon distributions and landscape traits such as stream size and depth.

This paper tests how differences in the distribution of salmon species across heterogeneous landscapes can influence the dispersal of salmon carcasses in riparian and estuarine habitats. Analyses interpreted how patterns in the distribution of live salmon, and the dispersal of dead ones, varied across coastal watersheds of the Northeastern Pacific that span a natural gradient in size, salmon density and other characteristics. First, this paper tested for differences in how live spawning pink (*O. gorbuscha*) and chum (*O. keta*) salmon were distributed within streams. Focus was on these two salmon species because they constituted more than 95% of total salmon within our study area, and thus are most important as a resource subsidy. It was anticipated that spawning pink salmon would concentrate in lower stream reaches, and chum salmon in upper reaches, potentially because larger chum salmon can successfully navigate higher gradients and spawn in larger substrate sizes of upper stream reaches within these coastal watersheds (Hunter, 1959; Scott and Crossman, 1973; Hale et al., 1985; Raleigh and Nelson, 1985). Second, patterns in salmon carcass transfer to riparian areas from adjacent spawning reaches by black and grizzly bears and by gray wolves were assessed. Based on initial field observations and on the aforementioned prediction, it was expected that wolf-transferred carcasses would concentrate in lower reaches of larger streams and therefore consist disproportionately of pink salmon. It was also expected that bear-transferred carcasses would occur throughout spawning reaches, particularly in upper portions of streams, across all stream sizes and consist mainly of chum salmon given the bears' preference for the larger size of chum salmon (Frame, 1974). Based on previous work, it was predicted that the magnitude of consumer-transferred carcasses would correlate positively with salmon density (Quinn et al., 2003) and negatively with stream depth due to reduced consumer access to spawning salmon (Andersson and Reynolds, 2017a). The findings presented in this study illustrate how species-specific responses, functional associations between species, and habitat traits can mediate the subsidy effects of salmon across coastal landscapes.

MATERIALS AND METHODS

Study Area

This study focused on 16 salmon-bearing watersheds within 45 km of Bella Bella (52°9′N, 128°8′W) on the central coast of British Columbia, Canada (**Figure 1**). This region lies within the

Coastal Western Hemlock biogeoclimatic zone and receives some of the highest levels of precipitation on the continent (Pojar et al., 1991). Landscapes in this part of North America remain largely intact due to their remoteness, restricted access, governance by First Nations, and support from conservation coalitions (Price et al., 2009).

Salmon Surveys

Live and dead pink and chum salmon were enumerated over a period of 5 years (2009–2013) between the months of August and October. Not all streams were surveyed every year (**Table A1**). Analyses were limited to pink and chum salmon as these species dominate our study region and account for >95% of total adult salmon spawners, with much smaller numbers of coho (*O. kisutch*) and a limited presence of sockeye (*O. nerka*) and Chinook salmon (*O. tshawytscha*).

For each site, only one live and dead salmon survey per year was used in analyses and occurred as close to peak spawning periods as time permitted. When more than one count was completed in a given year the count that had the highest number of live spawning pink and chum salmon combined was used in analyses. Peak spawning periods, when the total numbers of actively spawning salmon were the highest, were identified from sites where multiple live spawner surveys had been completed and from stock assessment surveys conducted by the Department of Fisheries and Oceans Canada and local First Nations fisheries programs in the same region. The first section counted in each stream was the spawning habitat in lower stream reaches below the highest extent of tidal coverage, which varied in length (**Table A1**). The remaining spawning areas upstream of the estuaries were divided into sections ranging 50–200 m in length depending on stream size, resulting in 3–10 sections per stream (excluding the tidal sections). Sections were measured in 50 m lengths or less using range finders accurate to the nearest meter. Streams were divided into sections starting at the stream mouth and ending at the upstream limit of salmon spawning to assess patterns in live salmon and carcass (bear and wolf-transferred) distributions within and between watersheds. Live spawning salmon were surveyed in an upstream direction and dead fish when returning back downstream. The entire spawning reach of each stream was surveyed. Most of these terminated at impassable barriers such as waterfalls or logjams. Sites that

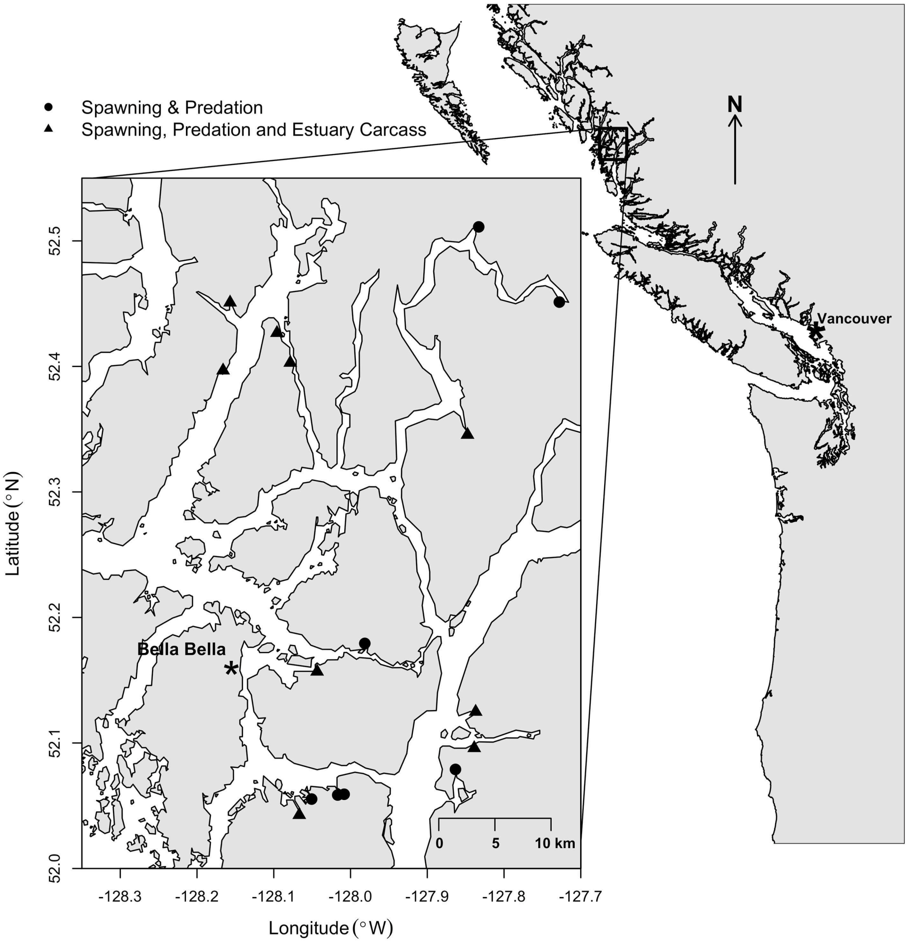

FIGURE 1 | Study area in the vicinity of Bella Bella on British Columbia's central coast. Circles indicate spawning and carcass transfer study sites from 2009 to 2013; triangles indicate where estuary salmon carcasses were also counted in 2008 and 2009.

did not have barriers to fish migration were surveyed upstream until there were no longer salmon present. Enumeration of live and dead salmon was by visual estimation from riverbanks when possible, and from within streams when bankside vantage points were not present (e.g., in canyons). Typically a 5–10 m length of stream was estimated at a time and totals were tallied once the end of a section was reached. At high densities, salmon were estimated in groups of tens to hundreds at a time and counted individually at lower densities. If weather conditions or turbidity prevented accurate enumeration, counts were omitted from analyses. Due to large differences in coloration and size, pink and chum salmon can easily be distinguished during counts. All crews were experienced in salmon enumeration and Fisheries and Oceans Canada (DFO) has integrated this spawner survey data into regional salmon escapement estimates. Variation in salmon body mass among different spawning populations was accounted for by weighing 5 dead adult salmon of each sex for each species from a subset of streams in our study region. Carcasses that were selected for weight were moribund or fresh pre-spawn mortalities. These mean salmon masses were applied to the remaining study sites that shared island groups, channels or mainland inlets to calculate carcass biomass.

Salmon carcasses were counted individually when possible and estimated in groups when necessary (e.g., bottom of large pools). Carcass categories were: senescent (spawned out), bear transferred (see below), wolf transferred, and unknown (**Table A2**). Carcasses were enumerated for each section of stream including a 10 m band of the riparian zone on either side of the stream channel for wolf- and bear-transferred carcasses, the riparian area known to contain the highest numbers of consumed carcasses (Cederholm et al., 1989). Senescent carcasses were identified as those that had no sign of consumption and were within stream channels or along banks. If the level of decomposition prohibited species identification or confirmation of consumption by bears or wolves, it was categorized as unknown.

Large differences between bear and wolf eating habits enable a considerable degree of certainty in determining which animal has consumed a salmon carcass. Bears consume multiple parts of a salmon including the brain, eggs and muscle tissue (Reimchen, 2000; Gende et al., 2004a), while wolves almost exclusively consume the brain in a surgical manner (Darimont et al., 2003). While these patterns may not be universal, they are supported by our own observations of active predation and scavenging within our study region (Field and Reynolds, 2013). Salmon carcasses that had been preyed upon or scavenged were categorized as follows: wolf-transferred carcasses were counted as having their heads or brains surgically removed, occasionally with parts of the jaws still attached (**Figure 2**), with no other part of the carcass consumed. Wolf-transferred carcasses could have smaller bite marks but lacked major rips and tears to the rest of the body. Bear-transferred carcasses were categorized as those that showed evidence of consumed eggs, bites and tears to body cavity and trunk muscle tissue, large bites or claw marks in the dorsal hump, and consumed brains (**Figure 3**, Andersson and Reynolds, 2017a,b). For carcass-transfer analyses (wolf and bear), carcasses were recorded as unknown and omitted from analyses when signs of consumption by bears or wolves could not be confirmed (including advanced states of decomposition). On average this comprised 36% of pink salmon carcasses and 22% of chum salmon carcasses across all streams and years.

FIGURE 2 | Wolf-transferred salmon carcasses with missing heads and minimal damage to body and trunk; no other parts of body consumed. Photo credit: Morgan Hocking (main), John Reynolds (inset).

Estuary Carcass Counts

In 2008 and 2009 intensive surveys were conducted for carcasses in the estuaries of a subset of nine of our study sites (**Table A3**). Intensive estuary surveys occurred separately from upstream counts because they were restricted to windows of low tide. All exposed carcasses and submerged carcasses to-2.4 m (below 0 m tide) were counted by species. Individual carcasses were counted whenever possible and estimated when there were large accumulations. For the latter, areas of carcass accumulations were measured and multiplied by mean carcass counts from several random 1 m^2 quadrats subsampled from the accumulation. Wolves and bears were not considered in estuary carcass analyses, as it was difficult to discern whether a carcass was actually consumed within an estuary or had been flushed downstream from upstream reaches.

FIGURE 3 | Bear-transferred carcasses with tears to body and trunk muscles tissue and eggs often consumed. Photo credit: Ben Rabinovitch (main), Morgan Hocking (inset).

Habitat Characteristics

Habitat data were not collected every year as many of these metrics do not change substantially year-to-year. Habitat measurements included the area of spawning habitat, average stream depth, and estuary meadow area. Data that do vary annually, such as stream discharge and salmon spawning density, were measured each year. Spawning area (stream size) was calculated as the total length of spawning habitat within a stream multiplied by the mean wetted width. Water depth and wetted width were measured at 12 random transects along a study reach selected within each site. Water depth was measured at 11 systematic locations along the length of each transect and wetted width was measured as the distance along each transect (from bank to bank along the water's surface). Each transect ran perpendicular to streams bisecting flow. Each habitat study reach length was determined by multiplying the mean stream bankfull width (mean width of the stream channel at its highest point before flooding banks) by 30 (Bain and Stevenson, 1999). Estuary meadow area was measured by sketching meadow habitat over aerial photographs and calculating areas using the Government of British Columbia's mapping website *iMap*BC (Government of British Columbia, 2006). Stream discharge was measured during each of the peak salmon-spawning periods of 2008 and 2009 at three randomly selected transects (of the 12 established per site) in each stream using a Flo-Mate 2000$^{\mathrm{TM}}$ portable flow meter. Flow measurements were recorded at 11 systematic locations along the length of each transect. Stream discharge, the cubic meters of water output per second, was calculated by multiplying stream flow by the cross-sectional areas of water at each transect location which was calculated from water depth measurements corresponding with each flow measurement location. Salmon densities were calculated as the total count of each salmon species divided by spawning reach or section area.

The number of wolves or bears was not determined at any of our sites. Similar to Quinn et al. (2003), this study was not examining the responses of wolves or bears to salmon density, but rather the spatial patterns of wolf and bear consumption of salmon and resulting subsidies to riparian areas. Although the number of consumers in a watershed would affect the total number of salmon transferred to riparian areas, these analyses were focused on the spatial patterns and overall magnitude of such transfers, including differences between wolves and bears, contrasts in subsidies of pink and chum salmon carcasses, and the relationships with basic physical characteristics of streams. It is possible our surveys may have influenced the natural behavior of consumers in these systems. However, most of these streams have been surveyed for years for salmon stock assessment purposes. In addition, surveys consistently covered the entire spawning length of each stream which limits the degree to which our presence might have biased the results.

Statistical Analyses

Generalized linear mixed-models were used to estimate the number of live spawners, wolf-transferred carcasses, and bear-transferred carcasses per section of stream. This accounted for the hierarchical structure (sections within streams) and non-normal distribution of count data (Bolker et al., 2009; Zuur et al., 2009). Models tested for the effects of distance upstream, stream size, and salmon species, including two-way interactions between all three variables. Dependent variables in these analyses were the number of live spawners, the number of wolf-transferred carcasses, and the number of bear-transferred carcasses per stream section for both chum and pink salmon. For wolf- and bear-transferred carcass analyses we also included salmon spawner density at the section level, average stream depth and total estuary meadow area. Analyses were conducted using the glmmADMB package in R (Skaug et al., 2010) using a negative binomial distribution with two random effects to account for intrinsic differences between watersheds and years. All models were competed using Akaike Information Criterion corrected for small sample sizes (AICc), which selects the most parsimonious model of the candidate set of models given the data. Zero-inflation parameters were included in live spawner and bear, but not wolf, analyses based on visual inspection of the data and the resulting lower AICc values of the global model (the model containing all covariates considered). All covariates were centered in all analyses to avoid inaccuracies in slope estimates for main effects as they can vary considerably depending on the presence of interaction terms (Schielzeth, 2010). A binary "dummy" variable was included in all analyses to investigate the differences between pink and chum salmon species ($0 =$ pink salmon, $1 =$ chum salmon) following the recommendations of Schielzeth (2010). Multicollinearity amongst all variables was generally low, with all variance inflation factors <3 and Pearson correlation coefficients <0.6 (Zuur et al., 2009, 2010). Goodness of fit was assessed using the coefficient of determination R^2. For live spawner and for wolf- and bear-transferred carcass analyses (GLMM) R^2 values are presented as both marginal (the proportion of variance described by fixed effects) and conditional (the proportion of variance described by both fixed and random effects; Nakagawa et al., 2017). For the estuary carcass analysis (GLM) a single coefficient of determination R^2 is presented as no random effects were considered (Tjur, 2009; Zhang, 2018). The open-source statistical software R was used for all analyses (R Core Team, 2016).

Multi-model approaches were used for wolf and bear analyses as top model weights were below 0.95 (Burnham and Anderson, 2002). For multi-model inference, models were constructed with scaled covariates (mean of 0 and standard deviation of 2) to enable direct comparison of effect sizes amongst covariates between wolf and bear analyses (Gelman, 2008; Grueber et al., 2011; Barton, 2012). Two stream section counts of live salmon were omitted from wolf and bear analyses due to leverage of the effects of salmon density (Crawley, 2007). These were the

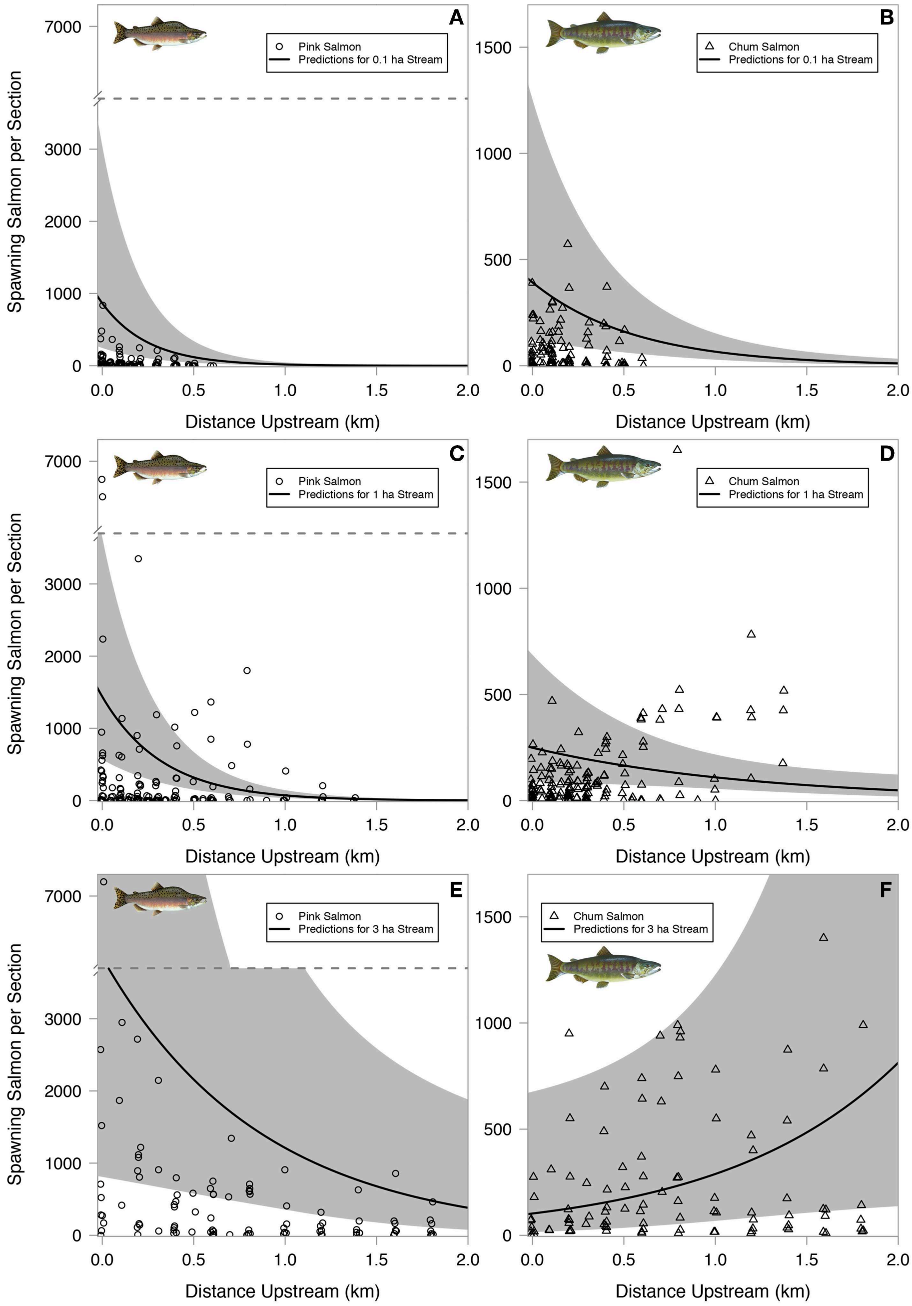

FIGURE 4 | Live chum and pink salmon distributions. Pink salmon in left column, chum salmon in right column. The top row **(A,B)** shows observed data points for small streams, defined as the lower 25th percentile of stream areas. The prediction lines are for the top model and are based on a stream with 0.1 ha spawning area. The middle row **(C,D)** illustrates a medium-sized stream, with observed data points between the 25th and 75th percentile of stream areas, and prediction lines for a 1 ha spawning area stream. The bottom row **(E,F)** shows observed data points above the 75th percentile of stream sizes with prediction lines for a stream with a 3 ha spawning area. Shaded polygons indicate 95% confidence bands around model predictions.

two highest values of pink salmon section densities (6.4 and 7 salmon/m^2; the range of remaining data for pink and chum salmon combined was 0–2.8 salmon/m^2). Candidate models were limited to the subset of models with a ΔAICc <4 (Burnham and Anderson, 2002). Parameter estimates for each variable were averaged across the candidate model set using the natural average method. Top model weights for both live spawner and estuary carcasses analyses were 0.99 and did not require model averaging.

To quantify the magnitude of salmon carcass inputs in estuaries, generalized-linear models were used to estimate the ratio of salmon that terminated in estuaries as carcasses for each watershed. The total abundance of salmon within a stream was used in this analysis as opposed to section-specific data. Estimates of the total number of salmon in each stream were generated by DFO using the area-under-the-curve method (AUC) from salmon counts conducted by the Heiltsuk First Nation, Simon Fraser University and DFO (Irvine et al., 1992). When insufficient counts were completed for AUC estimation, peak abundance estimates were used, which are strongly correlated with AUC estimates in these streams (Hocking and Reynolds, 2011). A binomial distribution was used for proportional carcass data and re-fit with a quasibinomial to address over dispersion observed in model residuals. This did not change coefficient estimates but did increase standard errors around the estimates. Models were ranked using AICc and quasi-information criterion for small sample sizes (QICc) for binomial and quasibinomial models, respectively (Lee and Nelder, 1999; Anderson and Burnham, 2002; Bolker, 2017). Both AICc and QICc model rankings and weights were identical.

RESULTS

In our study area, mean salmon weights in different streams for pink and chum salmon ranged from 0.9 to 1.3 kg and 2.5 to 3.6 kg, respectively. Over 5 years (2009–2013), a total of 718 sections of stream were surveyed for live and dead salmon. Salmon counts ranged from 0 to 7,200 and from 0 to 1,990 per section for live pink and chum salmon spawners, 0 to 333 and 0 to 22 for wolf-transferred pink and chum salmon carcasses, and 0 to 165 and 0 to 194 for bear-transferred pink and chum salmon carcasses, respectively. Estuary carcass counts (2008 and 2009) ranged from 0 to 21,909 and from 8 to 7,820 for pink and chum salmon

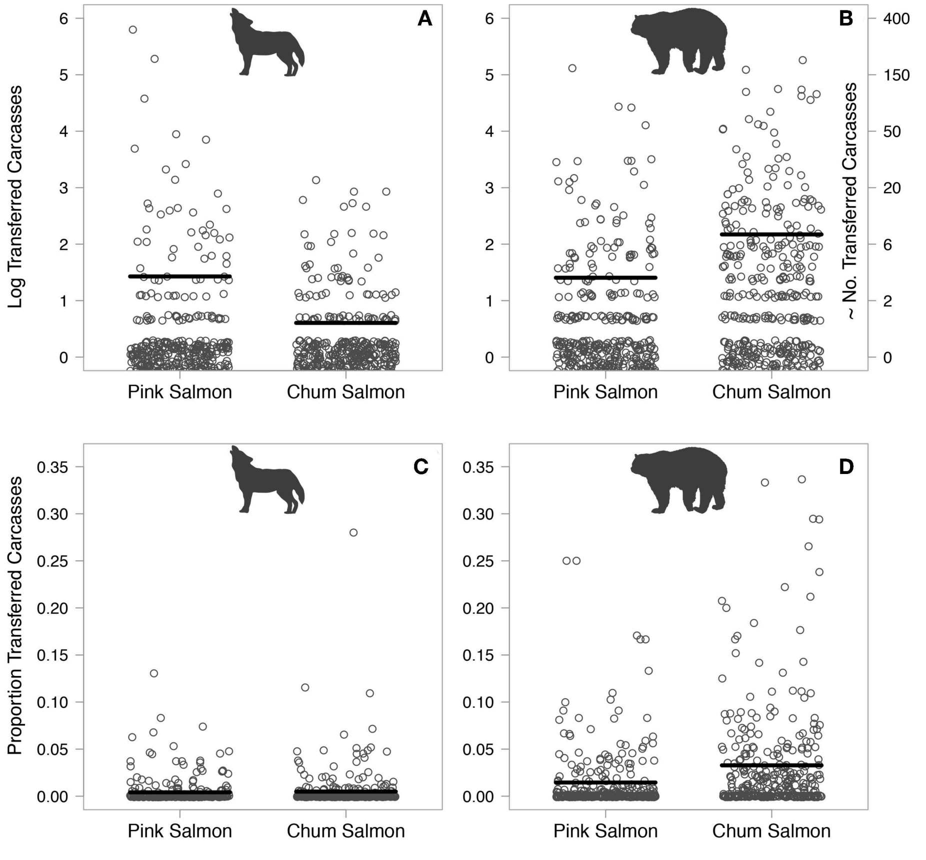

FIGURE 5 | Log-transformed counts of wolf- **(A)** and bear-transferred **(B)** carcasses for each salmon species per stream section. Proportion of total salmon consumed by wolves **(C)** and bears **(D)** per stream section. Proportions were calculated as the number of wolf- or bear-consumed carcasses relative to total salmon (sum of wolf and bear consumed, senescent and live) per stream section. Horizontal lines indicate mean values. Data points are jittered horizontally for display purposes. Gaps in data are a result of log-transformation.

carcasses, respectively. The supplementary material provides more detailed site-level summaries and model specifics.

Live Spawning Salmon

In small streams, both spawning pink and chum salmon were most abundant in lower stream reaches. However, as spawning area increased, pink salmon remained in lower reaches while chum salmon moved into upper stream reaches (**Figure 4**). The best model predicting spawner distributions had an Akaike weight >0.99, a marginal R^2 of 0.22, a conditional R^2 of 0.48, and contained all variables considered including distance upstream, total spawning area, and salmon species (**Table A4**).

Carcass Transfers to Riparian Areas

A total of 1,424 and 3,871 wolf- and bear-transferred carcasses were identified over 5 years, respectively. Wolves transferred over three times more pink salmon (1,125) than chum salmon (299) carcasses. Bears transferred approximately half the number of pink salmon (1,079) compared to chum salmon (2,792) carcasses (**Figures 5A,B**). The proportion of salmon carcasses transferred by wolves within each section did not vary by salmon species, and bears transferred higher overall proportions of salmon than wolves, in particular chum salmon (**Figures 5C,D**). Stream-level proportions of wolf-transferred carcasses ranged from 0 to 3.6% and from 0 to 8.1% for pink and chum salmon, respectively. Total proportions of bear-transferred carcasses ranged between 0 and 9.6% and 0 and 23.3% for pink and chum salmon, respectively. These estimates are low given the limited 10 m band of riparian area that was surveyed and the discounting of unknown carcasses. The number of wolf-transferred carcasses decreased with increasing distance upstream for both pink and chum salmon (**Figure 6A**). Bear-transferred pink salmon carcasses decreased similarly, but to a lesser degree than wolf carcasses, while bear-transferred chum salmon carcasses increased in upstream sections (**Figure 6B**). Salmon density had a much lower effect on the magnitude of wolf-transferred carcasses than those transferred by bears. Wolf-transferred pink salmon carcasses increased only slightly

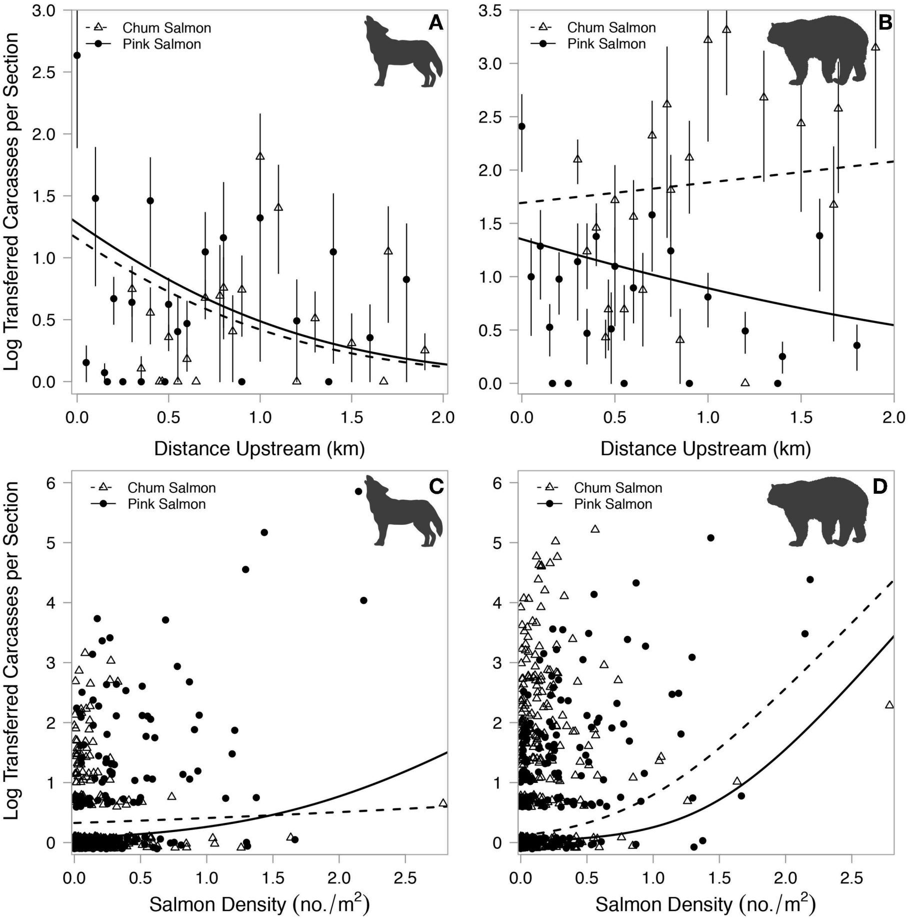

FIGURE 6 | Log-transformed counts of wolf- **(A)** and bear-transferred **(B)** carcasses for each salmon species per stream section with increasing distance upstream. Log-transformed counts of wolf- **(C)** and bear-transferred **(D)** carcasses for each salmon species per stream section with increasing salmon density. Data points are mean values with 95% confidence intervals for panels **(A,B)** and raw data points for **(C,D)**. Lines represent model predictions for each species with all other covariates held at mean values.

at higher pink salmon densities (**Figure 6C**). The number of bear-transferred carcasses for both salmon species increased with spawner density (**Figure 6D**).

The strongest correlates of wolf-transferred salmon were habitat characteristics; riparian areas adjacent to larger and shallower spawning areas and surrounded by larger estuary meadows contained the largest numbers of wolf-transferred carcasses (**Figure 7A**). The negative correlation between distance upstream and the number of wolf-transferred carcasses did not change with stream size but did strengthen at higher salmon densities (**Figure 7A**). For bear-transferred carcasses the negative correlation with distance was only notable when associated with pink salmon carcasses (**Figure 7B**). Bear consumption of salmon increased in streams with larger spawning areas but stream depth and estuary meadow area had negligible or uncertain effects (**Figure 7B**). General patterns suggest that habitat traits drive wolf transfer of salmon carcasses while salmon density and species drive patterns in bear carcass transfers to riparian areas. Akaike weights for the top wolf- and bear-transferred carcass models were 0.59 (marginal R^2 of 0.32 and a conditional R^2 of 0.50) and 0.10 (marginal R^2 of 0.17 and a conditional R^2 of 0.38), with candidate sets consisting of 3 and 30 models, respectively (Anderson and Burnham, 2002; Burnham and Anderson, 2002; **Table A5**).

Salmon Carcass Inputs Into Estuaries

The proportion of salmon carcasses that reached estuaries decreased with longer spawning reaches in streams. This negative correlation was stronger for pink salmon than chum salmon, opposite to our predictions (**Figures 8A,B**). The most parsimonious model describing the proportion of salmon carcasses in estuaries had a weight of evidence of 0.99, a R^2 of 0.67, and included upstream salmon density, spawning length, and an interaction between spawning length and salmon

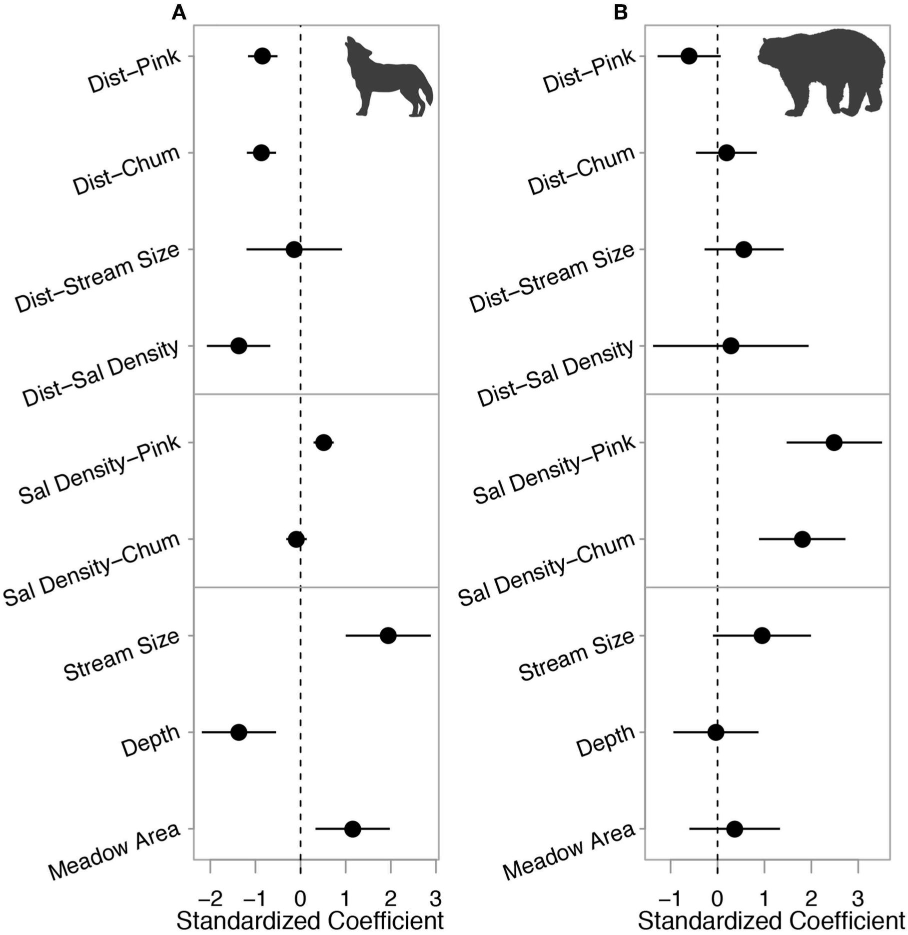

FIGURE 7 | Standardized coefficients (mean = 0, standard deviation = 2) with 95% confidence intervals for all covariates considered in the **(A)** wolf candidate model set and **(B)** bear candidate model set. Coefficient values indicate the average change in number of transferred carcasses per stream section as the associated covariate values increase by 2 standard deviations. Dist = distance upstream (km), Sal Density = salmon density (no./m^2), Stream Size = total spawning area (m^2). The top two rows show the relative effects of distance upstream on both wolf- and bear-transferred pink and chum carcasses as presented in **Figures 6A,B**. The two middle rows, isolated by horizontal lines, show the relative effect sizes of salmon density as presented in **Figures 6C,D**. Two variables separated by a hyphen indicate the effect of the interaction between those two covariates on wolf- or bear-transferred carcasses.

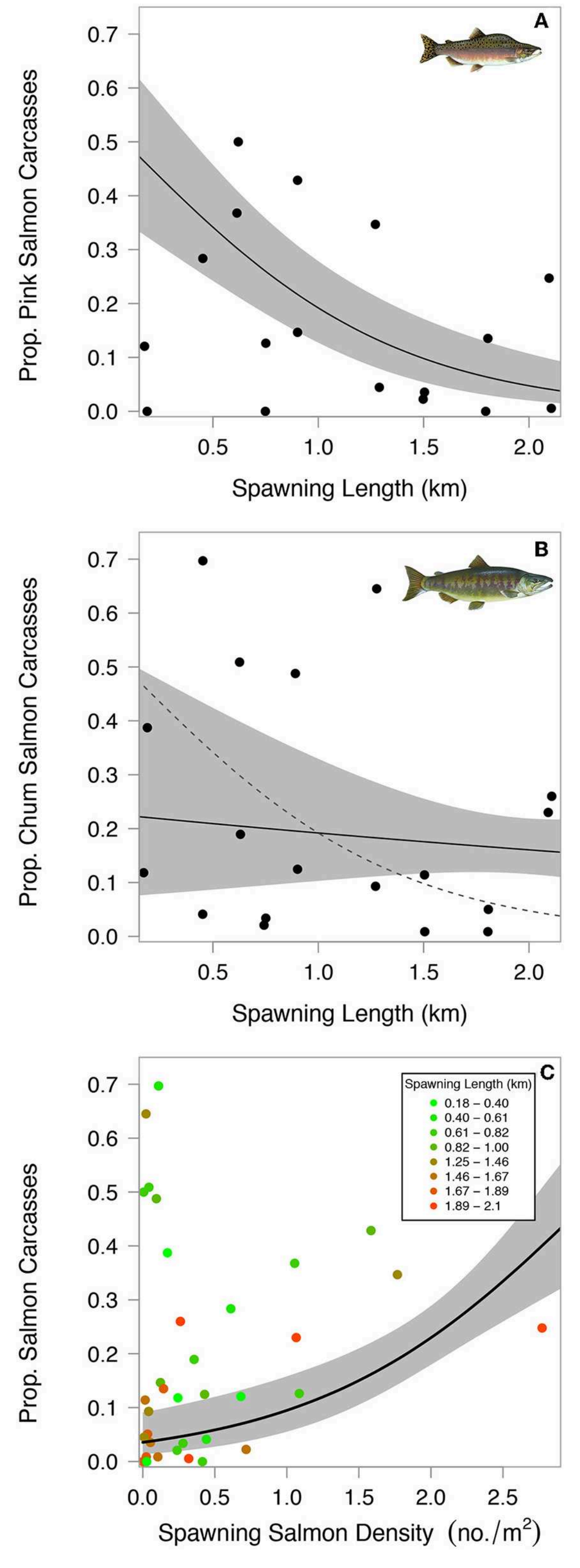

FIGURE 8 | Proportion of salmon carcasses in estuaries relative to total spawning channel length for pink salmon **(A)** and chum salmon **(B)**. Proportion of salmon carcasses in estuaries as a function of total salmon density **(C)**. Raw *(Continued)*

FIGURE 8 | data points are colored according to spawning channel length. Solid lines and shaded polygons show model predictions and 95% confidence bands with all other covariates held at mean values. The dotted line in panel B shows pink model trend line for visual comparison between salmon species, indicating higher proportions of chum carcasses end up in estuaries when the total length of spawning channel exceeds approximately 1 km.

species (**Table A6**). Stream discharge and year were absent from this model. Estuaries below streams with spawning reaches < ∼1 km had higher proportions of pink salmon carcasses while those below longer streams had higher proportions of chum salmon carcasses (**Figure 8B**). Higher upstream salmon densities increased the proportion of carcasses in estuaries. However, this relationship was related to the length of spawning reach (**Figure 8C**). Total numbers of estuary carcasses were highly variable between the 2 years. Carcass numbers, total carcass biomass, and biomass density (kg of salmon carcass per m^2) were much lower in 2008 than 2009, the latter of which was a high pink salmon-return year (**Figures 9A–C**). Chum salmon comprised the majority of 2008 carcass inputs into estuaries but pink salmon comprised the majority of carcasses in 2009, and for both years combined, even when correcting for differences in salmon size (carcass biomass) and estuary size (biomass density).

DISCUSSION

The distributions of live pink and chum salmon were mediated by stream size, with the two species diverging in spawning distributions as stream size increased. Although these salmon species share similar spawning habitat requirements (Nelson et al., 2015), larger chum salmon may not be as limited in terms of suitable spawning habitat types. The larger size of chum salmon may enable them to access and successfully spawn in reaches with more variable discharge (Neave, 1966a), higher water velocities (Hale et al., 1985; Raleigh and Nelson, 1985), and larger spawning-substrate sizes (Hunter, 1959; DeVries, 1997). Chum salmon may occupy upper reaches in larger streams as a density-dependent response to saturated habitat by pink salmon in lower reaches or because offspring can survive periods of high substrate scour in larger substrate sizes (Montgomery et al., 1996). Alternatively, higher peak discharge, or scour depths in upper reaches of larger catchments could constrain pink salmon to downstream areas. It is also possible that pink salmon prefer smaller particle sizes and plane-bed channels of lower reaches while chum salmon prefer upstream pool-riffle channel structures (Neave, 1966b; Montgomery et al., 1999). These contrasts in distribution suggest variation in subsidy potential among salmon species in adjacent riparian and estuarine landscapes, and have not been considered previously.

The expectation that there would be more wolf-transferred salmon in estuaries and lower stream reaches was validated, but the prediction that pink salmon would constitute the majority of wolf- transferred carcasses was not supported. Wolf transfer of salmon carcasses was strongly driven by habitat characteristics, such as shallow reaches of larger streams surrounded by large open meadows, and not solely by the density or species of salmon. This could be a result of habitat preference as salmon consumption by gray wolves has been observed to be concentrated in estuaries (Darimont et al., 2003, 2008). Habitat partitioning between wolves and bears may also explain this pattern as dietary partitioning has been documented among these terrestrial consumers (Merkle et al., 2017).

Bear-transferred carcasses showed a different pattern. Distance upstream correlated negatively with the number of bear-transferred pink salmon carcasses but showed a negligible relationship with chum salmon carcasses. Bears exhibited a strong foraging preference for chum salmon, possibly due to their larger size as suggested by Frame (1974). This bias could also be an artifact of the observation that bears consume salmon throughout stream reaches, of which chum salmon increasingly dominate in upper reaches of larger streams. Our analyses suggest that, on average, bears transfer more chum salmon than pink salmon carcasses to riparian zones throughout stream reaches, regardless of stream size. In addition, the disparities between bear-transferred pink and chum salmon carcasses in riparian areas increased with distance upstream. This, supported by previous work showing that bear carcass transfers are density-dependent (Quinn et al., 2003), suggests that bears are going to areas with the highest spawning salmon densities, particularly of chum salmon, and that stream habitat traits are of less importance. Our expected effect of stream depth differed from work in Alaska by Quinn et al. (2009), who reported larger percentages of carcass transfers in deeper streams. In our study, depth did not influence amounts of bear carcass transfers, but deeper streams did correlate with reduced numbers of wolf transfers. Our contrasting results could relate to differences in stream depth, fish distribution or other habitat characteristics between Alaska and British Columbia. Reductions in wolf carcass transfers may reflect their limited ability to catch salmon in deeper stream reaches. Andersson and Reynolds (2017b) also found that bears are more likely to selectively consume higher quality portions of salmon carcasses in narrow, shallow streams, which may play an indirect role in the amount of salmon material that is deposited in riparian areas to some degree.

Overall, the total quantity of salmon carcasses transferred to riparian zones, by wolves and bears combined, was highest in estuaries and declined upstream. This upstream decline was a result of decreases in wolf-transferred carcasses and corresponding reductions in the number of pink salmon carcasses transferred by bears. Therefore, the magnitude of salmon subsidies per stream section was roughly equal between salmon species in lower reaches, with contributions from both wolves and bears, but shifted to predominance of bear-transferred chum salmon as distance upstream increased. Overall, subsidy potential may be higher with chum salmon carcasses given their larger size, but this is contingent on the amount of carcass left by consumers. Somewhat surprisingly, a study in the same region by Andersson and Reynolds (2017b)

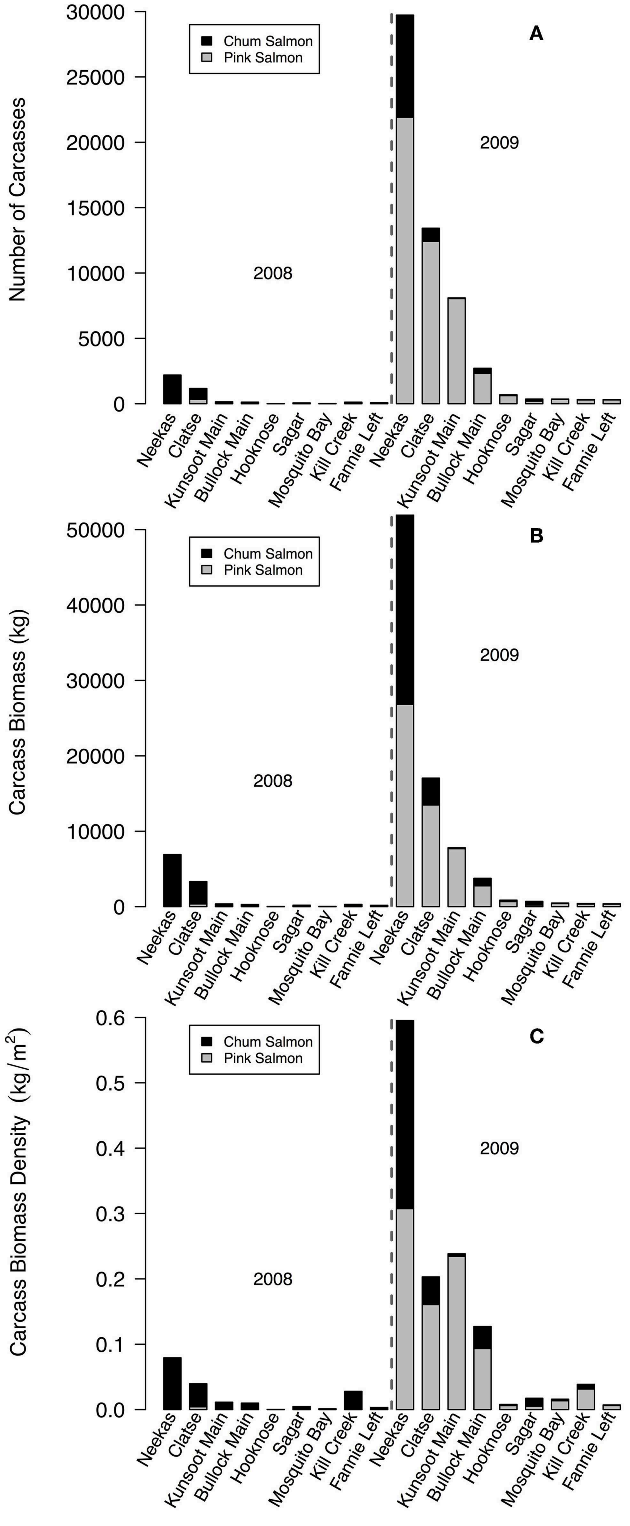

FIGURE 9 | Contributions of salmon carcasses to each estuary for 2008 (a low pink return year) and 2009 (a high pink return year), indicating **(A)** total number of carcasses; **(B)** total biomass of carcasses (kg); and **(C)** biomass density of carcasses (kg/m^2). Salmon biomass was calculated by measuring the weight of 5 dead adult salmon of each sex for each species in a subset of study streams covering our study area in 2009. Biomass densities are calculated for the total area of exposed estuary at a 0 m tide.

showed that selective consumption of carcasses by bears does not depend on the abundance of spawning salmon in these streams. Future studies that investigate how abundances of bears and wolves drive the magnitude of riparian salmon subsidies would be helpful. Studies that attempt to connect the amount of salmon transferred out of streams by consumers to how many carcasses ultimately reach estuaries, or consider differences in riparian subsides between streams that are dominated by grizzly bears vs. black bears, would also be of interest. Notably, the use of a 10 m-wide riparian band to assess carcass transfers is appropriate as the majority of carcass transfers are thought to occur within the first 10 m of riparian area adjacent to streams (Cederholm et al., 1989); however, bears also transport salmon farther into adjacent forests, and this distance can vary with the salmon density, fish freshness, and bear social hierarchies (Reimchen, 2000; Gende and Quinn, 2004; Quinn et al., 2009).

The prediction that salmon carcass inputs into estuaries were dominated by pink salmon as a result of spawning pink salmon concentrating in lower stream reaches was observed. However, the prediction that, as stream length increased, proportions of pink salmon carcasses in estuaries would remain relatively stable while the proportion of chum salmon carcasses would decrease was not supported by the data. Results showed that pink salmon carcass proportions decreased more than chum salmon carcasses as stream length increased. Although carcasses have been shown to travel short distances (Cederholm and Peterson, 1985; Cederholm et al., 1989; Minakawa and Gara, 2005; Strobel et al., 2009) high discharge events can cause longer distance carcass transport (Glock et al., 1980). Thus, high rainfall and variable discharge regimes in our study region, which were not necessarily captured by our spot flow measurements, may export larger proportions of carcasses from upstream reaches. However, lower than expected proportions of pink salmon carcasses below longer streams may be explained if portions of pink salmon carcasses are washed seaward out of intertidal areas due to their smaller size. Alternative salmon metrics, such as biomass per unit stream discharge, may be more appropriate for other salmon inputs such as particulate matter or dissolved nutrients (Johnston et al., 2004; Cak et al., 2008).

Total carcass inputs into estuaries differed greatly between the 2 years. This could be driven by differences in pink salmon abundances that cycle between even and odd years. In 2008, a low pink salmon-return year, carcass inputs were dominated by chum salmon and total numbers were much lower than the following pink salmon-dominant year. Over both years combined, total carcass inputs were dominated by pink salmon. This suggests that pink salmon populations could drive longer-term patterns in estuarine responses to salmon carcass subsidies (Harding et al., 2015). It is also possible that the comparatively prolonged spawning seasons of chum salmon could bias our carcass counts if the majority of chum salmon were still alive during surveys. However, the higher numbers of live pink salmon observed in lower reaches of these streams during surveys, and the fact that chum salmon generally start spawning earlier than pink salmon within our study region, support our confidence in these results (Neave, 1966a). Further, notable separation of spawning periods between salmon species was not observed within sites during data collection.

This study has demonstrated how the potential effects of salmon nutrient subsidies in coastal ecosystems vary within and across landscapes, by species of salmon, and through associations with major terrestrial consumers. Studies do not currently consider taxonomic variability in salmon-subsidy potential or spatial variability beyond average, site-level salmon density metrics and comparisons of above and below salmon migration barriers (Hocking and Reimchen, 2002; Mathewson et al., 2003; Harding and Reynolds, 2014b). Our analyses could provide a framework to guide future studies that investigate productivity responses to salmon subsidies in coastal systems, and specifically those that consider how subsidy effects on recipient ecosystems might be influenced by patterns in spawning salmon density and distribution in concert with the presence of terrestrial consumers and habitat characteristics. Such studies will further improve our understanding of complex, multi-scale ecosystem dynamics and processes. This work also highlights the importance of sound management decisions in the conservation and protection of salmon and populations of large terrestrial consumers to maintain ecologically important functional associations and mechanistic processes that link offshore marine productivity with coastal forests and estuaries (Chapin et al., 1997; Helfield and Naiman, 2006; Artelle et al., 2013).

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Canadian Council on Animal Care Guidelines, Simon Fraser University Animal Care Committee. The protocol was approved by the Simon Fraser University Animal Care Committee.

AUTHOR CONTRIBUTIONS

JMSH and JR conceptualized the study. JMSH, JNH, RF, JP, NS, and MW collected the data. JMSH conducted and interpreted analyses and drafted the paper. JNH, RF, JP, NS, MW, and JR critically reviewed and revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2019.00192/full#supplementary-material

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Understanding the Relative Roles of Salmon Spawner Enrichment and Disturbance: A High-Frequency, Multi-Habitat Field and Modeling Approach

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Organisms exert multiple, and often contrasting, influences on ecosystems. During their spawning runs, Pacific salmon (*Oncorhynchus* spp.) deliver nutrients to freshwater ecosystems, but also disturb benthic sediments during upstream migration and nest building. The relative importance of these contrasting roles is not well understood, especially in relation to the dynamics of other environmental drivers. To assess the influence of salmon-mediated enrichment and disturbance, we measured stream biofilm metrics (production, respiration, chlorophyll *a* [chl*a*], ash-free dry mass, stable isotope signatures, ∼ every 8 days) and stream variables (spawner and carcass abundance, dissolved nutrients, temperature, discharge, light, daily to every few days) from July through September (salmon arrived in August), in multiple habitats of a southeast Alaska (USA) stream. Biofilm production and biomass increased around the start of the salmon run, but declined later in the run. Biofilm stable isotope composition indicated incorporation of salmon-derived carbon and nitrogen (N) during the latter part of the run. Biofilm biomass differed among benthic habitat types (i.e., riffles, pools, stream edges) but temporal patterns were generally similar, suggesting that salmon and environmental influences were not habitat-specific. We used these high-frequency field data to parameterize an ordinary differential equation model for dissolved inorganic N, chl*a,* and cellular N, and estimated model parameters using Markov chain Monte Carlo. Posterior distributions indicated that (1) habitats and locations were generally similar in model parameters, (2) removing the effect of salmon resulted in no change in biofilm chl*a* early in the run (mid-August), but higher chl*a* biomass for some habitats later in the run (September), and (3) the overall integrated salmon effect over the run was one of biofilm loss. Only by combining high frequency biofilm and environmental data with a process-based model could we determine how environmental context dynamics interact with salmon run dynamics to modulate the biofilm response in natal spawning streams.

Keywords: marine-derived nutrients, epilithon, periphyton, environmental context, salmon runs, *Oncorhynchus*

INTRODUCTION

The physical and chemical template of ecosystems combined with biological processes provide the *environmental context* (after Janetski et al., 2009) that influences ecosystem structure and dynamics, including the response to resource subsidies (*sensu* Polis et al., 2004). Resource subsidies (Polis et al., 1997) take on many forms, such as organic matter provided to streams through leaf-litter fall (Wallace et al., 1997) or marine nutrients deposited on islands by birds (Anderson and Polis, 1999). Most research on resource subsidies has focused on the influence of material quantity versus quality (Marcarelli et al., 2011; Sitters et al., 2015) or the timing and duration of influence (Subalusky and Post, 2019). In fact, the influence of subsidies on a recipient ecosystem varies in relation to the available endogenous resources and the environmental context (Marczak et al., 2007; Subalusky and Post, 2019). Both the subsidy and environmental context can be spatially and temporally dynamic, creating complex patterns in responses, and currently limiting our understanding of the overall importance of resources subsidies.

Pacific salmon (*Oncorhynchus* spp.) are an important resource subsidy in their native range (Gende et al., 2002) and are often viewed as ecosystem engineers due to their pronounced influences on stream ecosystems (Moore, 2006; Flecker et al., 2010). Salmon provide nutrients through excretion and decomposition of their carcasses, but they also disturb the benthos by scouring substrates during redd construction. The nutrient enrichment and physical disturbance of the benthos can potentially drive the dynamics of benthic biofilm (i.e., the complex of algae, bacteria and fungi on submerged surfaces), whose responses may propagate throughout the entire food web and influence whole stream ecosystem dynamics. The net ecological effect of salmon on benthic biofilms is one of nutrient enrichment, that may enhance biofilm productivity, and its offset by benthic disturbance. In addition, the effect of salmon likely changes over time related to spatial and temporal dynamics in the size, number, and behavior of salmon (i.e., the subsidy dynamics) and also in the environmental context. For example, finer sediments are more easily disturbed (Tiegs et al., 2008; Janetski et al., 2009), and heavy canopy shading may prevent salmon enrichment (Ambrose et al., 2004). Understanding the effects of salmon is therefore challenging because it requires explicit quantification of the enrichment and disturbance effects in the context of subsidy and environmental dynamics.

Quantifying salmon enrichment and disturbance effects has been approached with field studies and modeling approaches. Salmon exclusion experiments have shown that disturbance of benthic biofilms due to nest excavation counteracts benefits to biofilm from nutrients excreted by salmon (Tiegs et al., 2011). However, such experiments cannot directly control for water-column nutrient enrichment, except by making comparisons between the periods before and during the salmon run, or to upstream reaches where salmon do not spawn. Each of these approaches has limitations, such as varying environmental conditions either in time or space that are confounded with the salmon run. Bellmore et al. (2014) developed a system dynamics model for biofilm, parameterized it with values from the literature, and performed simulations to address the dual roles of salmon in relation to the environmental context. They concluded that enrichment from the presence of salmon only occurs under specific environmental contexts, namely if background nutrient concentrations are low and the portion of the stream bed suitable for spawning is small. Most empirical studies on salmon effects have limited inferential power because they consist only of single sampling points before and during the run (e.g., Rüegg et al., 2012; Harding and Reynolds, 2014; but see Moore and Schindler, 2008), precluding parameterization of a dynamic model of salmon influence on benthic biofilms. In addition, studies of salmon subsidy effects often focus on stream riffles where salmon typically spawn and, therefore, exert the most disturbance (e.g., Tiegs et al., 2008, 2011). Other stream habitats that are less suitable to spawning (e.g., pools) remain understudied but may respond differently (Bellmore et al., 2014). To better understand the importance of salmon on the productivity of their natal spawning streams, we need to address habitat variability in response to salmon spawners and uniquely identify the salmon effects in relation to both subsidy dynamics (e.g., run size and timing) and other environmental variables (e.g., discharge, irradiance).

We evaluated the ecological influences of salmon spawners on biofilms in a southeast Alaska (USA) stream by combining high-resolution field data on salmon, biofilm, and environmental context with a dynamic process-based model. We measured multiple biofilm metrics approximately weekly across multiple locations for three different habitat types. We also surveyed salmon and environmental characteristics at least every few days, starting prior to and over the course of an annual salmon run. We then parameterized the model with the field data for all locations separately, and performed simulation experiments to infer separate enrichment and disturbance effects, and therefore the net salmon effect on biofilm.

MATERIALS AND METHODS

Study Site

We conducted our study in Twelve Mile Creek, Prince of Wales Island, in southeastern Alaska, USA. The Twelve Mile Creek watershed is 31 km^2 and our 300-m study reach had an average discharge of 1320 L s^{-1} (range, 25 to 6450 L s^{-1}) during the study (early July – late September 2008). Salmon spawners, which were dominated by pink salmon (*Oncorhynchus gorbuscha*) with low numbers of dog salmon (*O. keta*), typically appear in the stream at the beginning of August and their density peaks in early September (Rüegg et al., 2012).

We identified three habitat types that we predicted would differ in their response to salmon (see **Supplementary Figure S1**). First, pools were considered a low-disturbance location, especially at their upstream end, as salmon tend to spawn in faster flowing areas (e.g., riffles and pool tail-outs; Quinn, 2005). Second, riffle/run habitats were selected as high-disturbance areas based on flow and suitability as high-quality spawning habitat (for simplicity we will refer to this habitat type as riffle). Third, edges were habitats within 0.5 m of the water's edge on the sampling day, located adjacent to riffle habitats, and disturbance-prone as either potential spawning habitat at high discharge or due to emersion/drying at low discharge. For each habitat, we haphazardly selected three replicate locations (i.e., three separate pools, riffles, or edges) within the 300 m reach, for a total of nine sampling locations.

Environmental Context

We measured multiple biological, chemical, and physical variables to quantify the environmental context before and during the salmon run. Salmon spawners and carcasses were counted every 2–3 days in 4 m belt transects every 10 m along the 300 m reach during the salmon run (5 August to 24 Sept 2008) (Tiegs et al., 2009). Because the transects covered 40% of the stream benthic area (i.e., 4 m belt transects every 10 m along the stream), we multiplied the number of salmon counted by 2.5 to estimate total numbers (i.e., 100%), and then converted to density using reach area (length × mean width). Dissolved nutrient concentrations were measured from water samples collected at least weekly before (1 July to 4 August 2008) and during the salmon run (5 August to 20 September 2008). Samples were filtered through Whatman® GF/F filters and frozen at −20°C until analyzed in the laboratory. A Lachat QC-8500 Flow Injection Autoanalyzer (Lachat Instruments, Loveland, Colorado, USA) was used to determine soluble reactive phosphorous (SRP) with the ascorbic acid method and nitrate (NO_3^--N) with the cadmium reduction method. Ammonium (NH_4^+-N) was determined on a Shimadzu UV-1601 spectrophotometer (Shimadzu Corporation, Columbia, MA, USA) using the phenol-hypochlorite method (see Levi et al., 2011 for detailed section "Materials and Methods"). Water temperature (°C) was measured hourly using HOBO data loggers (Onset Corporation, Pocasset, MA, USA). Discharge (L s^{-1}) was estimated from water level changes recorded every 30 min by an Odyssey capacitance meter (Dataflow Systems, Inc., Christchurch, New Zealand) and a standard rating curve (Levi et al., 2011). Daily means were calculated for both temperature and discharge. Light intensity (μE cm^{-2} d^{-1}) was measured using an Odyssey light meter (Dataflow Systems, Inc., Christchurch, New Zealand) and integrated to obtain total daily photosynthetically active radiation (PAR).

Biofilm

Benthic biofilm was sampled every 8 days from 7 July to 20 September 2008. In each of the nine locations, we haphazardly collected three representative rocks touched blindly that fell within the gravelometer size classes of 32–90 mm. From these rocks, we measured net community production (NCP), community respiration (CR), gross community production (GCP = NCP + CR), chlorophyll *a* (chl*a*), and ash-free dry mass (AFDM). The three rocks per location were averaged and one value for each location was used for statistical analyses. Additionally, we measured the stable isotope composition (δ^{13}C and δ^{15}N) of biofilm using the aggregate of the three rocks per location.

In the field, we used a light/dark chamber method (Bott, 2006) to measure NCP and CR. Rocks were placed into 960-mL clear plastic cups (Mold-Rite Plastics, Plattsburgh, NY, USA). Cups were filled with stream water and closed underwater to eliminate air bubbles. Streamwater dissolved oxygen (DO) was recorded using a Hach Luminescent DO probe (Model HQ30d, Hach Company, Loveland, CO, USA) along with the closing time for each cup. Cups were then placed on the sediments and exposed to *in situ* light and temperature conditions. Light absorption by cups (37% of light available at the water surface) was similar to the absorption by stream water at depth of incubation (53%). After a minimum of 2.5 h, cups were opened and DO and time recorded. Water was then replaced with fresh stream water, cups closed, and placed in black plastic bags to simulate night-time conditions. Protocols followed those of the light incubations. Following the incubations, rocks were placed in individual plastic bags and transported to the laboratory in a cooler.

In the laboratory, each rock was scrubbed (all surfaces) with a stiff brush to measure chl*a* and AFDM. A known sub-sample of the resulting biofilm slurry was filtered onto a Whatman® GF/F filter. Filters were analyzed sequentially for chl*a* and AFDM using standard methods (see Tiegs et al., 2008). Chlorophyll *a* was determined fluorometrically after extraction in ethanol. Ash-free dry mass was determined after drying for at least 48 h at 60°C, followed by ashing for 3 h at 500°C. The remaining slurries of the three replicate rocks per location (e.g., pool 1) were combined, centrifuged, and dried at 60°C for analysis of stable isotopes. The dried biofilm was acidified to remove carbonates, redried, ground, and analyzed for nitrogen and carbon stable isotope composition using a Carlo Erba Elemental Analyzer coupled to a Finnegan Delta + Mass Spectrometer (Chaloner et al., 2002).

Scrubbed rocks were measured for length, width, and height; surface area of the entire rock was calculated assuming an ellipsoid. Water displacement by rocks in sampling cups was also measured. From the changes in DO and the volume of water used for incubations, we determined net community production (NCP; light incubation) and community respiration (CR; dark incubation) assuming a linear change and expressed rates per unit surface area (mg O_2 m^{-2} h^{-1}) (after Johnson et al., 2009; Rüegg et al., 2011). Gross community production was determined as the sum of NCP and CR fluxes for a specific rock. Due to low CR relative to NCP, GCP and NCP were very similar and only GCP and CR were used in statistical analyses. Chlorophyll *a* and AFDM were also calculated on a per unit area basis (mg m^{-2} and g m^{-2}, respectively). Stable isotopes are presented as their isotopic ratios (δ^{15}N, δ^{13}C). Isotopic analyses also provided percent nitrogen in samples (see modeling below).

Statistical Analyses

To examine habitat and time effects on biofilm metabolism, biomass, and stable isotope composition, we used a generalized additive model (GAM; Zuur et al., 2007). We treated habitat type as a fixed factor with three levels: pools, riffles, and edges, as we expected them to differ in their biofilm characteristics. We used Julian day as the continuous time variable, as we expected biofilm characteristics to change over the course of the study period, in response to changes in the environmental context (i.e., seasonal trends in light, temperature, salmon run dynamics).

We used a smoother for the time variable as data followed non-linear patterns. We also included interaction effect between time and habitat type as we expected the influence of salmon and potentially other environmental characteristics to be habitat-specific, and the temporal patterns to differ among the habitats [GAM model: Dependent variable ∼ Habitat type + s(Julian Day) + s(Julian Day, by = Habitat Type)]. The response variables were the six biofilm metrics (GCP, CR, chl*a*, AFDM, δ^{15}N, and δ^{13}C) and we included the specific location (nine locations) as random effects (*N* = 90). We used the GAM analyses to support the description of temporal patterns detected while we use the dynamic model presented below to detect various aspects of the salmon effects and make predictions about what would have occurred without salmon (for the chl*a* metric). All analyses were conducted using R 2.11.2 (R Core Team, 2019), with the mcgv package used for the GAM models.

Modeling Biofilm Dynamics in Relation to the Environmental Context

We examined the influence of salmon density on biofilm accrual (as inferred from chl*a* concentrations) using a process-based model to disentangle the salmon influence from changes in the other environmental characteristics, such as discharge which is generally higher during salmon runs in our study system. As in Bellmore et al. (2014), who developed a single equation for chl*a* dynamics, our objective was to model the major influences on the dynamics of DIN, N concentration in benthic biofilm, and chl*a* with established formulations describing these processes. We explicitly modeled the linked dynamics of inorganic nitrogen (N) in the water column with biofilm N and chl*a* to predict inorganic N and chl*a* in the absence of salmon. Our main goal was to capture the influence of salmon and abiotic variables (i.e., light, temperature, and discharge) on biofilm chl*a* in a reasonable fashion by estimating parameters for different locations in the stream, and therefore wanted our model to be flexible enough to match location-specific dynamics. The stream system modeled here showed nutrient limitation and its alleviation by salmon in a previous year (Rüegg et al., 2011), as well as location-specific differences in sediment size and thus potential salmon disturbance (e.g., Tiegs et al., 2008; Rüegg et al., 2012), allowing us to address our goals.

The dynamics of dissolved inorganic N (i.e., DIN, N in the equations), chl*a*, and N in the biofilm (i.e., N_B in the equations) are described in eqs. 1–3. Here, the units for N are nitrogen mass per unit volume (m^3) or concentration in the water column, whereas the units for chl*a* and N_B are mass per benthic area (m^2). To derive the dynamics of DIN concentration, we considered a parcel of water over 1 m^2 of benthic area and treated the flow into and out of the parcel as a chemostat (Smith and Waltman, 1995). We used a mass balance approach, incorporating chemostat-like advective flow of DIN into and out of the water column (Smith and Waltman, 1995), and Michaelis-Menten uptake kinetics (e.g., Kim et al., 1990, 1992) to describe the loss of DIN from the water column due to algal uptake. We assumed that salmon-derived DIN is proportionally to salmon density. Combing the chemostat-like advective inputs and losses, the salmon fertilization effect, and DIN removal by biofilm, we arrive at,

$$\frac{dN_{mass}}{dt} = \frac{Id\left(1m^2\right)v}{1m} - \frac{Nd\left(1m^2\right)v}{1m} + \eta S\left(1m^2\right) - \frac{\alpha_N\left[chla\right]N}{K_N + N} \tag{1a}$$

in which the first two terms describe changes in DIN mass due to advection, the third term describes the salmon subsidy to the DIN pool, and the fourth term prescribes DIN uptake by biofilm as a saturating function of DIN (see eq. 2 below). The first two terms can be combined to yield a simpler expression,

$$\frac{dN_{mass}}{dt} = (I - N)\frac{d\left(1m^2\right)v}{1m} + \eta S\left(1m^2\right) - \frac{\alpha_N\left[chla\right]N}{K_N + N} \tag{1b}$$

In eqs. 1a and b, *I* represents the input from upstream DIN, and *N* is the DIN concentration in the column, both of which are multiplied by the volume of the water parcel (*d* = depth multiplied by the 1 m^2 benthic area) and by discharge (*v*). Again, this component of eqs. 1a and 1b corresponds to advective flow. The salmon nutrient subsidy of N into the DIN pool, the second term in eqs. 1a and 1b, is the product of salmon density (*S*) for 1 m^2 benthic surface area, and the rate of N production via excretion and decomposition (η). Biofilm uptake of DIN, the third term in eqs. 1a and 1b, is described by a saturating Michaelis-Menten function of DIN, with the maximum uptake rate, α_N, with units of N mass per unit chl*a* per unit time. Thus, total DIN losses from the parcel above the 1 m^2 benthic area result from advection moving DIN downstream and from uptake by biofilms, whereas inputs result from upstream flow and salmon. To model DIN concentration on an areal basis, we divided the entire equation by the water parcel volume (i.e., 1 $m^2 \times$ d) to obtain the equation for DIN concentration in the parcel,

$$\frac{dN}{dt} = (1 - N)v + \frac{\eta S}{d} - \frac{\alpha_N}{d}\frac{\left[chla\right]N}{K_N + N} \tag{1c}$$

To model biofilm dynamics, we employ an approach similar to Bellmore et al. (2014), assuming that chl*a* production adheres to Liebig's law of the minimum. Specifically, the maximum production rate (μ) is multiplied by the minimum of Droop functions of light (as described by PAR_{min}) and cellular nitrogen (as described by N_{Bmin}). We added temperature-dependence of chl*a* production by multiplying the production rate by an Arrhenius function, as in Brown et al. (2004) (E_a = activation energy, κ = Boltzman constant, and *T* = water temperature in Kelvin), while chl*a* loss occurs due to flow or salmon-induced disturbance and other mortality (*m*) (eq. 2). We assumed that disturbance-related loss was a threshold phenomenon (i.e., critical discharge for biofilm loss, Q_{crit}) and used a sigmoidal function of discharge, the Hill function, in which β controls the steepness of the threshold (e.g., Eggert et al., 2012). We also assumed that salmon density (S) could be linearly translated into discharge (γ) so that salmon disturbance effects could be directly incorporated into the flow-induced loss of chl*a* from the benthic surface underlying the water column. Combining all these effects on chl*a* production yields the following equation for chl*a* dynamics,

$$\frac{d\left[chla\right]}{dt} = \mu\left[chla\right]\left[MIN\left[\left(1-\frac{PAR_{min}}{PAR}\right)\left(1-\frac{N_{Bmin}}{N_B}\right)\right] e^{\frac{-E_a}{\kappa T}} - m - \frac{(Q+\gamma S)^{\beta}}{(Q+\gamma S)^{\beta}+Q_{crit}^{\beta}}\right] \qquad (2)$$

To link the dynamics of DIN with the growth of biofilm, we require a third equation describing the dynamics of intracellular N (i.e., cell quota) because chl*a* production is explicitly a function of the N quota (Legović and Cruzado, 1997). Cellular N quota increases as a function of uptake from the water column, and decreases as a function of chl*a* production rate, described by,

$$\frac{dN_B}{dt} = \frac{\alpha_N}{K_N+N} - \mu MIN\left[\left(1-\frac{PAR_{min}}{PAR}\right)\left(1-\frac{N_{Bmin}}{N_B}\right)\right] e^{\frac{-E_a}{\kappa T}} N_B \qquad (3)$$

in which the first term describes cellular uptake from DIN in the water column and the second term describes loss from the biofilm N pool.

We used the discharge, light, temperature, and salmon density data collected over the course of the study to drive the dynamics of the above model, by linearly interpolating each between the observed data points. For a given set of parameters, we simulated the above system to obtain trajectories for all three state variables (N, chl*a*, N_B) and assumed that differences between the simulated (model predicted) trajectories and the observed data were the result of independent and identically distributed normal observation error at each time point. We used uniform priors for all parameters (I, η, α_N, K_N, PAR_{min}, N_{Bmin}, E_a, m, β, γ, and Q_{crit}), and used an adaptive random walk Metropolis-Hastings algorithm (Haario et al., 2001) using the metrop() function in R to obtain the posterior distributions of all the parameters. Multiple Markov chains were run for several hundred thousand iterations for each location, and trace plots were visually inspected to ensure convergence and stationarity. Posterior distributions were generated from traces that were thinned after an initial burn-in period. Separate models were fit to each of the locations to account for potential differences among habitats and locations within habitats. To assess the enrichment, disturbance, and net effects of salmon, we simulated the model for all sets of parameters from the joint posterior, but with salmon abundance set to zero for the entire duration of data collection. We then created envelopes defined by the 2.5th and 97.5th quantiles (i.e., 95% confidence interval), for each location and state variable combination in the presence and absence of salmon. This allowed us to use the uncertainty in parameters that resulted from variability in our measurements to infer statistical significance of salmon effects. Thus, spans of time during which trajectory envelopes for salmon and no-salmon do not overlap correspond to times when the salmon effect is unlikely to occur by random chance. Furthermore, model parameters were used to estimate the seasonal gain of chl*a* biomass and loss (integration under modeled curves), as well as N uptake, again both in the presence and absence of salmon spawners.

RESULTS

Environmental Context

The first live salmon (primarily pink salmon, *O. gorbuscha*) in Twelve Mile Creek were observed on 5 August, and abundance then increased to >1000 salmon in the 300 m reach within 2 weeks, before declining near the end of September (**Figure 1A**). Salmon carcasses began to accumulate in the stream channel on September 5 and were nearly as abundant as live spawners at the end of September (>300 carcasses in the 300 m reach).

Nutrient concentrations increased concomitantly with salmon presence. Soluble reactive phosphorous (SRP) concentrations increased from fairly constant levels prior to and during the initial stages of the run (~5 μg L^{-1}) to much higher levels as carcasses became very abundant (up to 28 μg L^{-1}; **Figure 1B**). Ammonium concentrations increased from fairly constant and low levels of ~2 μg L^{-1} prior to salmon arrival to over 20 μg L^{-1} within 2 weeks of the arrival of live salmon spawners (**Figure 1C**). Toward the end of the run (September), ammonium concentration declined with live salmon abundance. Nitrate concentrations also increased in the presence of salmon, but more slowly than ammonium concentrations and peaked at 250 μg L^{-1} in mid-September (**Figure 1D**).

Physical conditions in the stream varied during the study. Streamwater temperature varied from 8.0 to 11.6°C with no pronounced association with salmon abundance (**Figure 1E**). Discharge varied from 25 to 3825 L s^{-1} before the salmon run, with most of the high discharge attributable to four events of >2000 L s^{-1} (**Figure 1F**). Discharge during the run generally remained below 2000 L s^{-1}, at a slightly higher base flow than before the run, with four high flow events exceeding 5000 L s^{-1} occurring during the early run (mid- to late-August). Light ranged from 20 to 470 μE cm^{-2} d^{-1} before the salmon run compared with 20 to 237 μE cm^{-2} d^{-1} during the run, reflecting the transition from summer to autumn (**Figure 1G**).

Biofilm

Biofilm functional metrics of gross community production (GCP) and community respiration (CR) showed clear temporal patterns (GAM Time effect, $p < 0.001$) and were consistent among the riffle, edge, and pool habitat sampled (GAM Interaction n.s.). Before the salmon run, GCP was relatively constant (7.2 to 9.6 mg O_2 m^{-2} h^{-1}), but increased shortly before the arrival of salmon and remained higher early in the salmon run (9.1 to 13.6 mg O_2 m^{-2} h^{-1}; **Figure 2A**). As the salmon run

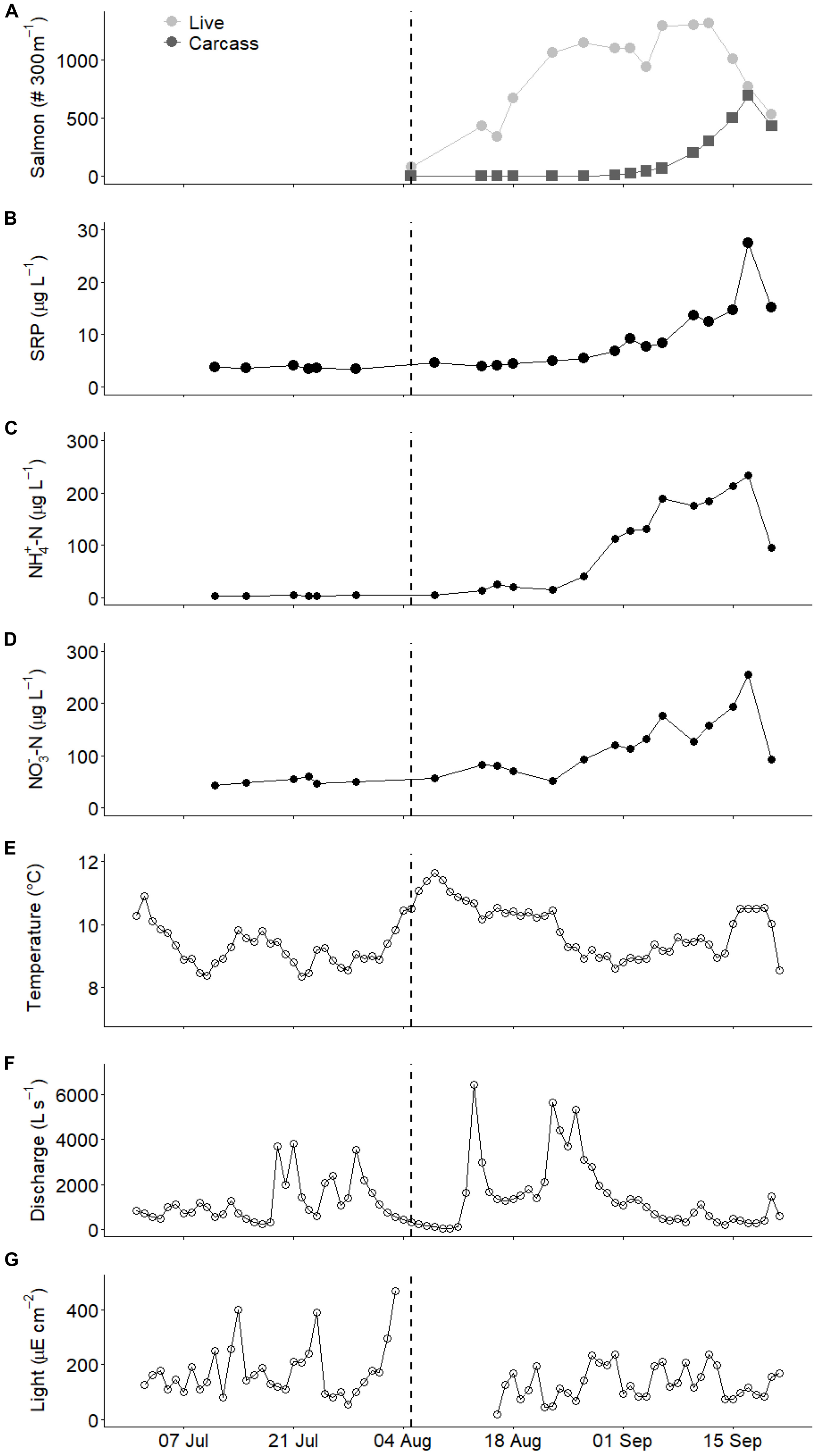

FIGURE 1 | Temporal patterns in environmental variables during the study period: **(A)** Salmon spawner and carcass abundances, **(B)** soluble reactive phosphorous (SRP), **(C)** ammonium (NH_4^+), and **(D)** nitrate (NO_3^-) concentrations, **(E)** streamwater temperature, **(F)** discharge, and **(G)** light intensity. Panels **(E–G)** are shown as mean daily values. Dashed vertical line represents the arrival of salmon spawners.

progressed, production decreased to the lowest recorded levels (0 to 6.0 mg O_2 m^{-2} h^{-1}). Respiration was low and at times undetectable (range −0.01 to 0.02 mg m^{-2} h^{-1}) without a clear seasonal pattern (**Figure 2B**). None of the metrics differed among habitat types (GAM Habitat n.s.).

Temporal patterns in biofilm biomass, namely chlorophyll *a* (chl*a*) and ash-free dry mass (AFDM), reflected those of production (GAM Time effect $p < 0.001$), but habitat types differed in biomass (GAM Habitat $p < 0.001$). Biofilm chl*a* increased over time before the salmon run (ranges among habitats: 1.8 to 6.5 mg m^{-2}) and reached the highest levels early in the salmon run (3.7 to 7.6 mg m^{-2}; **Figure 2C**). As with GCP, chl*a* declined rapidly to levels lower than before salmon (0.3 to 2.1 mg m^{-2}) later in the run. Chlorophyll *a* was greater in pool habitats than in edge and riffle habitats (**Figure 2C**). Biofilm AFDM patterns were similar to chl*a*, ranging from 0.7 to 1.4 g m^{-2} before the salmon run, 1.2 to 1.9 g m^{-2} during early in the salmon run, and 0.5 to 1.2 g m^{-2} later in the run (**Figure 2D**). Pools had the greatest AFDM. Temporal patterns did not differ among habitats. Differences among habitats did not change over time for either chl*a* or AFDM, except for AFDM biomass in pools which increased and then decreased more rapidly than in the other habitats (GAM Time × Pool Habitat Interaction, $p = 0.022$).

Biofilm stable isotope ratios varied significantly over time (GAM Time effect $p < 0.001$) and showed some variation among habitat types. Nitrogen stable isotope ratios were relatively constant before the salmon run (δ^{15}N 1.0 to 2.4‰) but increased shortly after the arrival of salmon (δ^{15}N 3.0 to 4.5%; **Figure 2E**). However, in all habitat types δ^{15}N then declined to pre-salmon levels (1.6 to 2.4%) for 3 weeks before increasing again toward the end of the salmon run (3.6 to 4.7%), with signatures similar among habitat types (GAM Habitat effect n.s.). Carbon stable isotope ratios were relatively constant before and early into the salmon run (δ^{13}C −33.3 to −30.5%) before increasing to relatively constant levels (−28.5 to −27.3%) by the end of September (**Figure 2F**). Habitats were significantly different (GAM Habitat type $p < 0.001$), likely due to the fact that each habitat showed a significantly different temporal pattern (GAM interaction between Time effect and pool, riffle, and edge habitat type all $p < 0.001$) with edge habitats increasing earliest, followed by riffles, and pools with the slowest C isotopic enrichment.

Influence of Environmental Context on Biofilm

Model parameters corresponding to maximum biofilm growth rate (μ), salmon enrichment rate (η), and discharge-based disturbance (Q_{crit}) did not vary systematically across habitat types, but some locations, rather than habitats, appeared to stand out. For the contribution of salmon to dissolved nitrogen (N), one location of each habitat (η of Pool 3, Riffle 2, Edge 2) was higher than average across all locations (**Table 1**). Biofilm growth rates were similar among habitat types and locations, as were minimum light and nutrient requirements. One notable exception was the minimum N quota of edge habitats, which had both the highest (Edge 1) and lowest modal values (Edge 2). Two locations showed some resistance (Pool 3, Riffle 2) to discharge disturbance, as evidence by higher values of Q_{crit}. Surprisingly, salmon disturbance effects, as indicated by the conversion factor (γ), were consistent across all locations. Also, the salmon-based disturbance effect was unrelated to the critical discharge, which would be expected if sediment stability were the most critical factor for salmon disturbance. Model parameter values suggest that variation in biofilm response to salmon response is not related to common habitat types but is the result of dynamics occurring at relatively small scales.

Model trajectories for all state variables approximate the observed dynamics well (**Figure 3** and **Supplementary Figure S2**), suggesting that the model does a reasonable job of capturing the essence of the biofilm-environmental context relationship. Our main goal in the modeling was to use reasonable formulations of the most important factors influencing biofilm growth to recreate the dynamics of DIN, chl*a*, and biofilm N. Using a model that is a reasonable reflection of reality and parameterized with field data allowed us to separate the salmon effect from the remaining environmental context by simulating the dynamics of the three state variables in the absence of salmon. Chlorophyll *a*, which is proportional to biofilm mass, exhibited short windows of increased accrual or loss with the presence of salmon. Removing the salmon effect, by setting salmon abundance to zero for the entire duration of data collection and simulating location-specific (in terms of parameters) models, indicated certain periods when biofilm accrual could exceed biomass expected if salmon were absent during a few days early in the run (around mid-August) (**Figure 3**). However, the generally overlapping confidence envelopes show that this enrichment effect is typically weak or non-existent. On the other hand, certain periods exhibited significantly lower biomass than expected if salmon were absent, based on the absence of overlap of the 95% confidence envelopes for trajectories in the presence and absence of salmon. These apparent disturbance effects by salmon spawner presence were evident in almost all habitat types and locations in September. Thus, the balance between salmon enrichment and disturbance may shift from minimal enrichment earlier in the run to net disturbance later in the run.

Comparing seasonal accrual and loss of biofilm chl*a* and N uptake indicated that salmon presence acted predominantly as a source of disturbance during the study in our stream reach. Biofilm chl*a* produced over the study was similar whether salmon were present or absent, suggesting that environmental variation beyond salmon presence may swamp salmon enrichment effects (e.g., decreasing light later in the season/run) (**Figure 4A**). Salmon clearly increased the loss of biofilm chl*a*, as losses in the presence of salmon were much higher than those in the absence of salmon (**Figure 4B**). While the uptake of nitrogen per unit chl*a* was much higher in the presence of salmon spawners than in their absence (**Figure 4C**), the higher biomass losses due to salmon disturbance limited the absolute amount of nitrogen retained in the stream ecosystem. Together, these results suggest that salmon have minimal enrichment effects, and that disturbance predominates, especially later in the run, which translates into a net negative cumulative impact on biofilm over the course of the run.

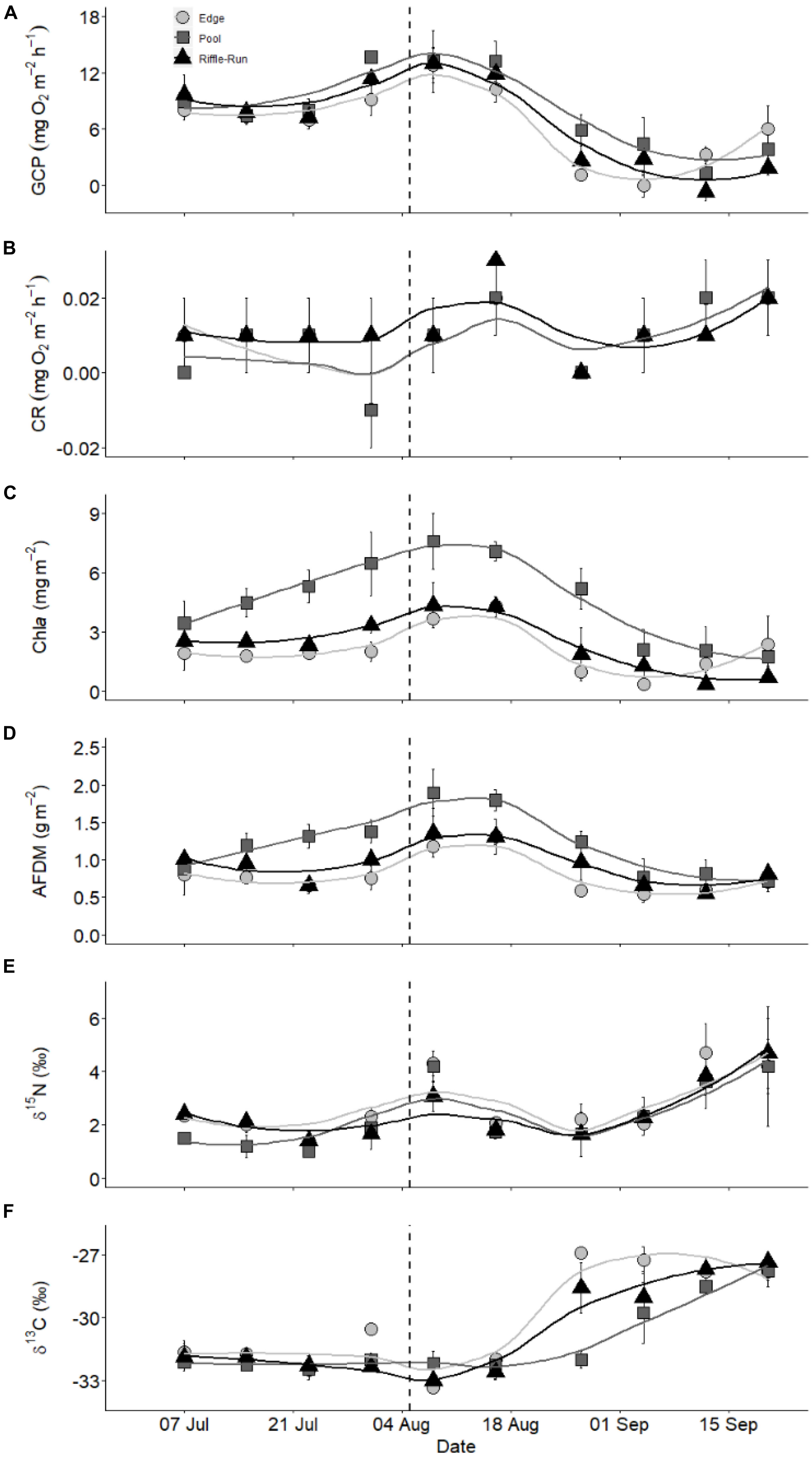

FIGURE 2 | Temporal patterns of biofilm characteristics across three habitat types (■ pools, ▲ riffles, ● edges): **(A)** gross community production (GCP), **(B)** community respiration (CR), **(C)** chlorophyll *a* (chl*a*), **(D)** ash-free dry mass (AFDM), **(E)** nitrogen stable isotope ratio ($\delta^{15}N$), and **(F)** carbon stable isotope ratio ($\delta^{13}C$). Dashed vertical line represents the arrival of salmon spawners. Trend lines were smoothed with loess (locally weighted smoothing).

TABLE 1 | Mode and *confidence interval* (5th to 95th percentile) of parameter value distributions.

Location		η	μ	PAR_{min}	N_{Bmin}	Q_{crit}	γ
Pool	1	30	4	22	12	18	69
		20–41	*0–9*	*0–47*	*7–16*	*8–28*	*21–99*
	2	26	2	31	8	17	66
		13–39	*0–6*	*0–57*	*1–16*	*4–28*	*18–100*
	3	53	4	20	11	54	64
		37–64	*0–9*	*0–46*	*9–12*	*20–93*	*31–100*
Riffle	1	30	2	23	11	16	65
		19–42	*0–4*	*0–71*	*2–23*	*7–25*	*27–100*
	2	80	0	30	11	47	53
		29–133	*0–1*	*1–66*	*0–27*	*15–89*	*17–100*
	3	31	3	19	10	17	66
		23–41	*0–8*	*0–43*	*5–15*	*7–27*	*26–100*
Edge	1	31	2	25	31	16	64
		19–40	*0–7*	*0–63*	*2–77*	*7–26*	*22–98*
	2	84	1	57	4	20	55
		27–151	*1–2*	*30–72*	*1–8*	*6–36*	*29–99*
	3	31	4	17	11	17	65
		23–40	*1–9*	*0–37*	*8–15*	*7–27*	*23–99*

η is the conversion of salmon abundance to nitrogen increases and thus represents the salmon enrichment effect (similar to the slopes of nitrogen increases with salmon abundance). μ represents the maximum biofilm growth rate as chla. PAR_{min} ($\mu E\ cm^{-2}\ d^{-1}$) represents the minimum light requirements for growth of chla. N_{Bmin} represents the minimum nitrogen required for growth. Q_{crit} represents the critical discharge, estimated statistically as the inflection point of the Hill equation, for loss of chla biomass due to abrasion. γ represents the conversion of salmon disturbance to equivalent disturbance due to discharge, and thus represents the strength of salmon disturbance.

DISCUSSION

Our high-frequency sampling approach, encompassing the biological response metrics and the environmental context, combined with a dynamic process model allowed us to address the question of the relative importance of salmon nutrient subsidies and physical disturbance on biofilm dynamics over the course of a run and across multiple locations within a stream. The magnitude of the salmon resource pulse (*sensu* Anderson et al., 2008) depends on the abundance of spawners and carcasses, which varies over time based on run dynamics. However, environmental conditions can also interact with the salmon run dynamics to modify the biotic responses to this nutrient pulse (Janetski et al., 2009; Subalusky and Post, 2019). For example, sediment size can strongly influence the extent of spawner-mediated enrichment versus disturbance (Tiegs et al., 2008; Holtgrieve et al., 2010) and nutrient limitation determines subsidy use (Rüegg et al., 2011). Theoretical models have supported the role of the environmental context (Bellmore et al., 2014), and spatio-temporal variation in stream environmental context could thereby modulate the ecological responses to salmon-mediated enrichment and disturbance (Rüegg et al., 2012; Subalusky and Post, 2019). However, no previous study has combined a high-frequency field data set such as ours with a dynamic model to separate the dual salmon effects from the general environmental context. Our model identified a small net disturbance effect of salmon that was fairly universal across habitat types. Pronounced salmon effects only occurred during small windows of time (a few days to 2 weeks), as evidenced by biofilm biomass being higher or lower than expected had the salmon run not occurred in the given environmental context. In other words, the effects of salmon as a keystone organism (*sensu* Willson and Halupka, 1995), be it as a resource subsidy or a physical disturbance (Gende et al., 2002; Moore, 2006; Flecker et al., 2010), may be most intense during limited periods for algal and nutrient dynamics related to species phenology such as timing and size of runs or spawning requirements (e.g., sucker, Childress and McIntyre, 2016). However, a sequence of salmon runs by different species, as occurs in many systems, may have an aggregate effect on ecosystem structure and function (Lamberti et al., 2010).

In Twelve Mile Creek, temporal changes in environmental context had stronger influences than did spatial difference among habitats (cf. Flecker et al., 2002; Geddes and Trexler, 2003), potentially due to changes to the stream context that lead to pervasive spawning throughout the reach. Namely, sediment sizes were generally smaller throughout the entire stream than those of similar streams with comparable run sizes in the area (Tiegs et al., 2008; Rüegg et al., 2012), reducing differences among the selected habitat types. Large salmon returns, as witnessed for pink salmon (*O. gorbuscha*) in Twelve Mile Creek, can force spawners to use all available space, resulting in spawner-mediated disturbance in lower-quality spawning habitat (Quinn, 2005). We realize that this study represents one season in a 300-m stream reach of one stream in southeast Alaska. Unfortunately, we were not able to extend the sampling past the end of the salmon run, but an earlier study in the region suggests that biofilms in some streams recover from salmon-mediated disturbance (Tiegs et al., 2008) and that salmon nutrients provide a limiting resource (Rüegg et al., 2011) that can persist beyond the actual run (Reisinger et al., 2013). Before-during comparison of salmon effects in this stream were consistent over multiple years (Rüegg et al., 2012), suggesting that the model may be applicable beyond the temporal scope of the study. Unreplicated study designs can provide valuable information on potential mechanisms, such as the one applied to this stream reach, but their applicability to other streams needs to be tested in further high-frequency studies. However, despite simplifications in both the study design and the representation of the environmental context (e.g., environmental characteristics only measured at reach scale), we were able to evaluate the relative roles of salmon enrichment and disturbance on biofilms. Our integrative approach indicates that salmon contribute more to biofilm losses than enrichment and, therefore, that their overall effect may be that of an ecosystem disturbance (Moore and Schindler, 2008; Bellmore et al., 2014). However, the short windows of enrichment may still be critical for overall stream ecosystem productivity, especially if salmon nutrients are integrated and propagated in stream food webs (e.g., macroinvertebrates or fish) and persist past the presence of salmon spawners (e.g., Reisinger et al., 2013; Harding and Reynolds, 2014).

Small-scale temporal responses of biofilm were evident for all metrics. Biofilm production, biomass, and nitrogen

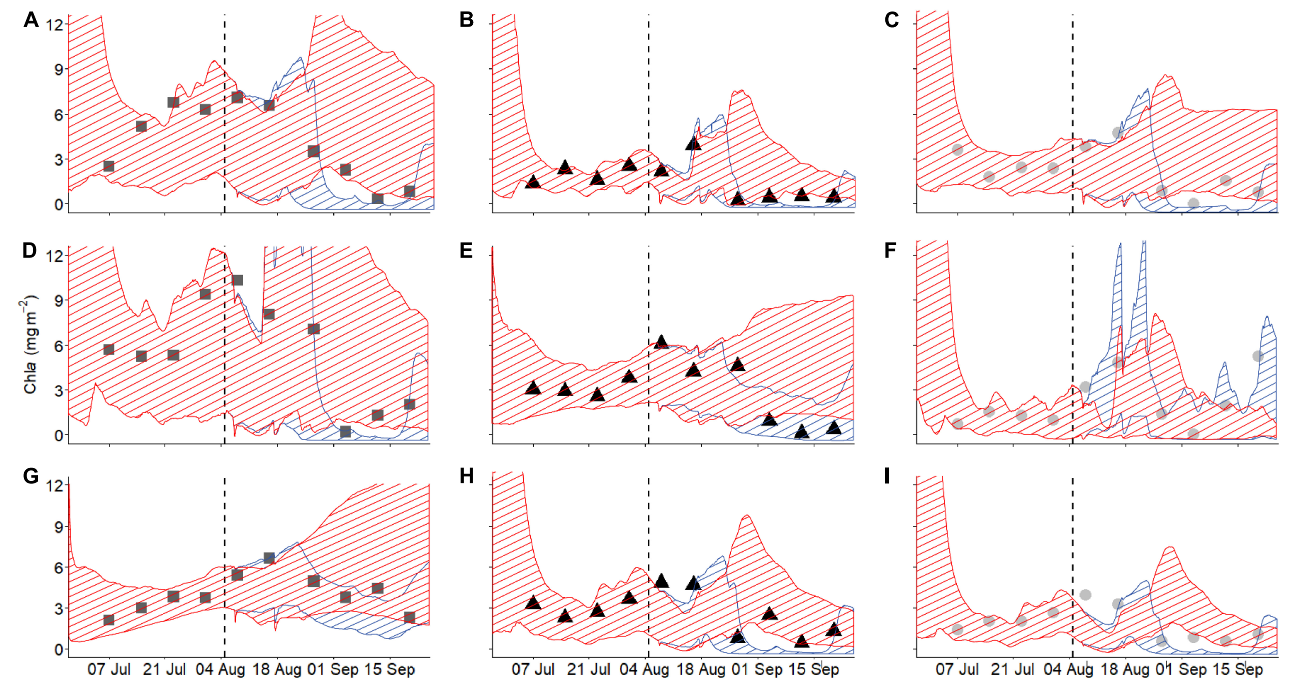

FIGURE 3 | Modeled (shaded area) and measured (symbols) biofilm chl*a* over the course of the salmon run at the nine habitat locations studied. Different columns show the different habitat types [left column **(A,D,G)** = pools (■), middle column **(B,E,H)**] = riffles (▲), right column **(C,F,I)**] = edges (●)], while the rows depict the three locations studied for each habitat type [top = 1 **(A,B,C)**, middle = 2 **(D,E,F)**, bottom = 3 **(G,H,I)**]. Blue shaded area indicates the confidence interval of the model including salmon while the red shaded area indicates the model with salmon abundance artificially set to zero (i.e., salmon were stopped).

isotopes increased early in the run. Biofilm exhibited rapid $\delta^{15}N$ enrichment (within a few days) similar to the isotopic signature of salmon material (cf. Chaloner et al., 2002), likely reflecting uptake of salmon-derived nitrogen by otherwise nutrient-limited biofilms (Rüegg et al., 2011). The absence of increased streamwater nutrient concentrations during that same period may be due to rapid uptake of salmon nutrients by biofilms. Later in the run, the increased dissolved nitrogen concentration due to salmon may have saturated the capacity for biofilm uptake (Levi et al., 2011; Rüegg et al., 2011). Most biofilm metrics exhibited sharp changes a few weeks into the salmon spawning run when the beginning of spawning activity would have scoured biofilm from sediments, thereby negating any enrichment effects of salmon (i.e., no net biofilm response; cf. Molinos and Donohue, 2010). This disturbance prevailed until the end of the study when disturbance dominated over enrichment and prevented biofilm biomass recovery. However, stable isotope signatures (i.e., isotopic enrichment) suggest that biofilms were actively growing while taking up salmon-derived dissolved nutrients during these periods of disturbance, assimilating more nitrogen per unit biomass than early in the run. Thus, salmon provide resources over the entire run, but physical disturbance may negate most of the enrichment effect at high salmon spawner densities and widespread disturbance (cf. Moore and Schindler, 2008). However, biofilm with low biomass but rapid turnover rates can still take up salmon nutrients and transfer that production to higher trophic levels (Lamberti and Resh, 1983; Morley et al., 2016), suggesting that enrichment needs to be defined both in terms of productivity and the amount of resources incorporated by direct (e.g., nutrient uptake by biofilms, consumption of eggs by resident fish) and indirect pathways (e.g., trophic transfers).

Our results show that salmon spawner effects may manifest at different time points of the salmon run, but that biofilm responses were similar with and without the presence of salmon over most of the run, supporting the theoretical findings of Bellmore et al. (2014) that an early period of enrichment is followed by predominantly disturbance later in the run, while the integrated effects of salmon are relatively balanced. Biofilm production can respond rapidly (within days to 2–3 weeks) to changes in environmental conditions (Biggs, 1996), potentially before live salmon abundances peak (3–4 weeks in this study). Thus, environmental conditions in the 1–2 weeks prior to sampling might be more critical determinants of biofilm responses than conditions at the time of sampling (cf. Holtgrieve and Schindler, 2011), potentially explaining why environmental conditions at the time of sampling often have low explanatory power (e.g., Rüegg et al., 2012). Salmon run dynamics may have contributed to observed patterns as salmon spawners reached peak densities within 2 weeks of the start of the spawning run. The period leading up to peak densities corresponds to when males that excrete nutrients but do not dig nests in sediment (Quinn, 2005) arrive en masse. Females that both excrete nutrients and excavate redds, thus disturbing the sediment, attained peak density after approximately 4 weeks; this potential ecological effect of sequencing of male and female

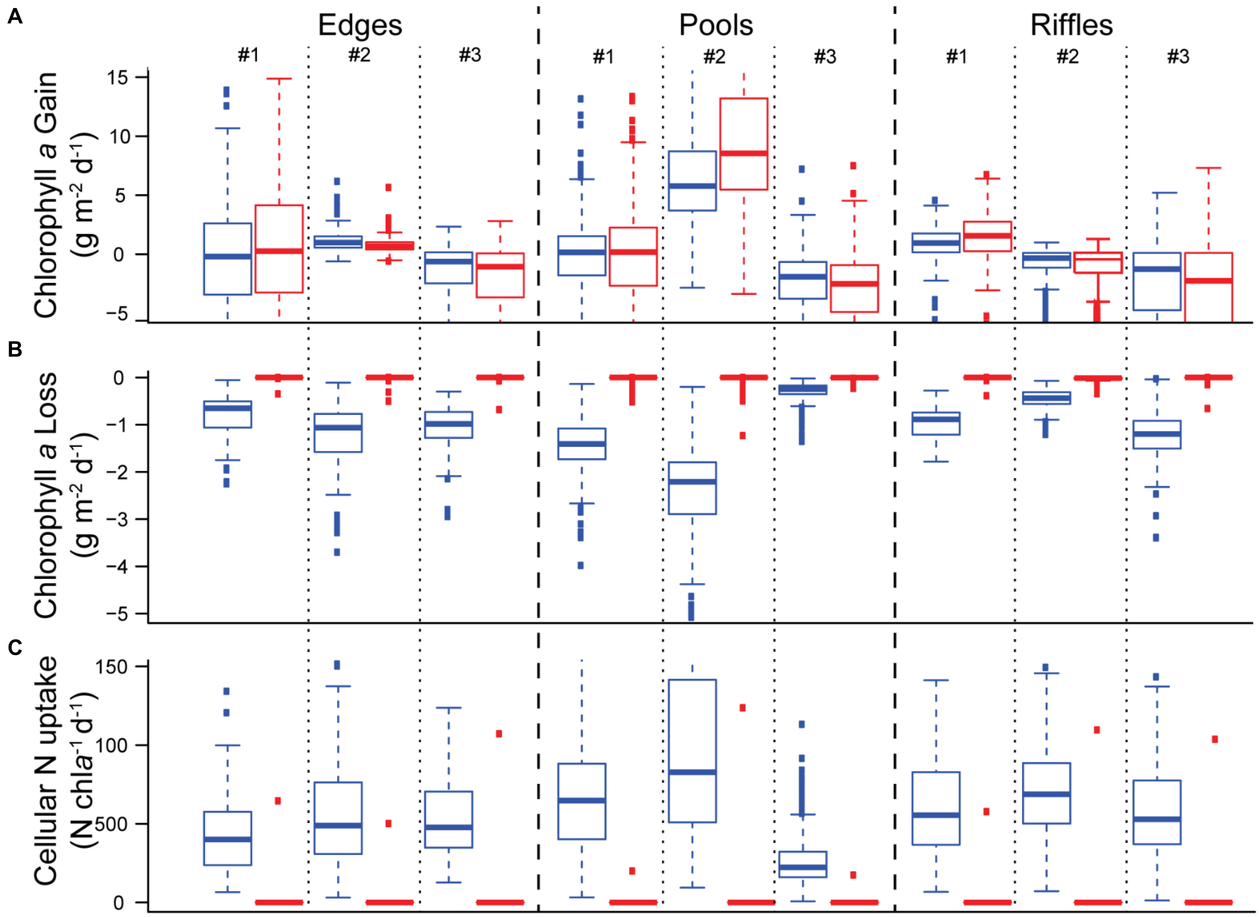

FIGURE 4 | Boxplots showing: daily **(A)** chl*a* production, **(B)** chl*a* losses, and **(C)** nitrogen uptake per unit chl*a* over the course of the run. Blue boxes indicate modeled values including the presence of salmon while red boxes indicate modeled values where salmon were excluded by setting salmon abundance to zero (i.e., salmon were stopped).

salmon has not yet been considered. The early part of the salmon run likely provides enrichment but little disturbance, which enables biofilm to respond with increased productivity during a time when other environmental conditions are also favorable (Bellmore et al., 2014).

An early window of subsidy use suggests that seasonal changes over the course of the run, such as declining light and temperature, may have little effect on the magnitude of salmon subsidy use because maximal response to salmon enrichment happens early in the run. Delayed arrival of male salmon into streams (Quinn, 2005), such as when low discharge prevents upstream migration, may therefore have important consequences because the period of net enrichment may decline relative to spawning disturbance. Our study suggests that spawner enrichment and disturbance are restricted to a narrow time window of about 6 weeks in this stream, whereas environmental factors such as flood disturbance can persist much longer (e.g., 3–6 months in coastal Alaska streams, Oswood et al., 2006). However, over the course of the run we infer that biofilm accrual is balanced by the dual effects of salmon, as daily biofilm growth was similar for model trajectories with or without salmon. Biofilm losses were clearly driven by the presence of salmon while nitrogen uptake increased in the presence of salmon, suggesting that despite the overall biomass loss, relative nutrient enrichment may still occur (e.g., Holtgrieve et al., 2010; Reisinger et al., 2013). While the relative importance of salmon enrichment and disturbance has been studied (e.g., Moore et al., 2004; Tiegs et al., 2009), this has rarely involved differentiating between enrichment and disturbance at daily time steps over the salmon run. Determining the salmon spawner effect with only limited sampling frequency may miss critical "windows of effects" or overemphasize the magnitude of the effect, especially considering the large spatial variability in responses among ecosystems (Chaloner et al., 2004; Tiegs et al., 2008; Rüegg et al., 2012).

Previous studies have rarely considered dynamic environmental variables, such as discharge and temperature (but see Chaloner et al., 2007; Rüegg et al., 2012), that could enhance or diminish salmon effects (Tiegs et al., 2011). A recent framework on animal subsidies argues that the context of the donor and recipient ecosystems determines the quality, quantity, timing, and duration of the resource subsidy, which also modulate the ecosystem's response to the subsidy (Subalusky and Post, 2019). Our field and modeling results suggest that considering salmon run dynamics in conjunction with dynamics of other environmental characteristics is key to understanding salmon as a subsidy to their natal streams. Variation in biofilm production and biomass during the salmon run is likely due to the synergistic effects of several environmental variables (cf. Wipfli et al., 1999; Hill et al., 2011), including increasing water temperature, low discharge, and high irradiance during high biofilm biomass periods (i.e., baseflow) at the onset of the salmon run. Our model further suggests that concurrent changes in light, temperature, nutrients, discharge, and salmon abundance all contribute to biofilm dynamics, and have implications for the interpretation of salmon effects (cf. Stevenson, 1997; Hill et al., 2011). Quantifying only the subsidy effect will overestimate salmon's positive bottom-up influence given their countering role as agents of disturbance. Considering the multiple roles of salmon, in conjunction with environmental conditions, is therefore needed to accurately predict the net impacts that salmon have on their natal streams.

CONCLUSION

By combining high frequency field data with a process-based model, we showed that changes in environmental context can interact with salmon run dynamics to modulate the response in stream biofilm. Early in the run, salmon nutrient enrichment was favored by low discharge, increasing temperature, and high light that enhanced biofilm. Later in the run, high discharge events combined with intense spawning activity and declining temperature and light slowed biofilm recovery, leading to a reduction in biofilm biomass. Overall, the net effect of salmon on benthic biofilm accrual was slightly negative, and driven by late-run disturbance. Our study demonstrates that the resource subsidy and sediment disturbance imparted by salmon are dynamic and interactive over the course of a salmon run. Thus, studies that target the period of peak spawner abundance are unlikely to capture the full variation in biologically important responses, be they those of basal resources such as biofilm or higher trophic levels, even if undertaken over a broad spatial scale encompassing multiple salmon streams. As such, high frequency sampling in multiple streams may be needed to sufficiently capture the complex ecological influence of salmon spawners on streams ecosystems.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request from the corresponding author.

AUTHOR CONTRIBUTIONS

JR, DC, and ST conceived and designed the study with inputs from JT and GL. JR, PL, and ST performed the sampling. JR and PL analyzed all samples. JR, CS, and FB analyzed the data. JR and DC wrote the manuscript. All authors provided major inputs and revisions to the manuscript.

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MINI REVIEW
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Contaminant Biotransport by Pacific Salmon in the Great Lakes

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In the Laurentian Great Lakes, introduced Pacific salmon (*Oncorhynchus* spp.) deposit resources and contaminants as carcass and gametic tissue during spawning migrations to tributaries. Such ecosystem linkages can increase growth and contaminant bioaccumulation in stream-resident fish but mechanisms driving this process remain unclear. In this mini-review, we synthesize findings from observational, experimental, and modeling studies related to Pacific salmon contaminant biotransport in the Great Lakes. First, contaminant biotransport varies among Great Lakes basins suggesting that basin-level characteristics including salmon abundance and historic contamination are important factors controlling the movement of contaminants from the lakes to tributaries. Second, stream-resident fish exposed to salmon have 24-fold higher PCB but moderately lower Hg concentrations when compared to locations without salmon. This finding is explained by differential bioaccumulation of PCB and Hg into different tissue types; analysis of salmon tissue indicates that eggs have elevated PCB and lower Hg than carcasses. Third, stream-resident fish exhibit a dietary shift and increased ration reflecting salmon egg consumption. Last, models suggest that salmon egg consumption can drive a trade-off between PCB and Hg bioaccumulation. This review identifies mechanisms controlling the transfer of salmon-derived energy potential strategies for management. Future research should be directed at identifying other biovectors and determining a list of emerging contaminants that could be subject to biotransport.

Keywords: Pacific salmon, contaminant biotransport, bioaccumulation, resource subsidy, ecosystem linkage

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MECHANISMS OF CONTAMINANT DISPERSAL

Pollution is one of five global drivers of environmental change that contributes to biodiversity loss and human health impairment (Assessment, 2005). Contaminants including persistent organic pollutants (POPs), heavy metals, and emerging contaminants including per- and polyfluoroalkyl substances (PFAS), pharmaceuticals, and microplastics are a concern to organisms, ecosystems, and human health (Murphy et al., 2012). Although environmental concentrations of some POPs (e.g., PCBs [polychlorinated biphenyls], DDE [1,1-dichloro-2,2-bis[p-chlorophenyl] ethylene], PBDEs [polybrominated diphenyl ethers]) have declined due to environmental regulations in the United States and later internationally under the Stockholm Convention, concentrations in fish remain high enough to warrant sport fish consumption advisories and to elicit concern over effects on piscivorous wildlife (Murphy et al., 2012). Risks to humans and wildlife persist due to the resistance of these pollutants to degradation coupled with their ability to bioaccumulate and biomagnify in food webs (Clements et al., 2012). The impact of contaminants can be magnified when contaminants are moved between disconnected ecosystems.

Understanding the movement of contaminants requires characterization of physical, chemical, and biological processes which dictate contaminant fate in the environment. Transport of contaminants has largely been described through mechanistic models of contaminant volatilization, deposition, and uptake dynamics (Mackay and Fraser, 2000). These models are useful for raising awareness of the risk and consequences of long-range contaminant transport to human populations, such as indigenous human populations in the Arctic (Blais, 2005). However, the biological aspects of contaminant transport in these models can be overly simplistic. For instance, bioaccumulation models often make predictions that inadequately describe an organism's lifetime consumption and growth, which strongly influences overall contaminant burden and concentration. In addition, they often fail to use site-specific environmental, diet, and contaminant data (Schiesari et al., 2018). Moreover, most models do not include biological processes, such as animal migrations that disperse contaminants across ecosystem boundaries (Kallenborn and Blais, 2015).

Conventional models of contaminant transport do not include biological transport of contaminants across ecosystem boundaries (Schiesari et al., 2018). Animal migrations are responsible for the mass transport of nutrients and energy (Bauer and Hoye, 2014). Indeed, these often predictable pulses of energy from migratory organisms strongly influence multiple trophic levels in the ecosystem receiving the subsidy (Polis et al., 2004; Lamberti et al., 2010), modulating ecosystem stability and community biodiversity (Bauer and Hoye, 2014). Relative to the subsidy effects, the process and broader impacts of contaminant biotransport remain poorly understood (Schiesari et al., 2018). Migratory organisms can accumulate and disperse large contaminant loads, but the mechanisms driving the spatial patterns and rates of bioaccumulation of biologically transported contaminants have not yet been synthesized. With this mini-review, we will describe the process of contaminant biotransport, using Pacific salmon in the Laurentian Great Lakes as a case-study to describe factors controlling contaminant biotransport. The Laurentian Great Lakes are useful for this task because of the large amount of information that has been generated on contaminants, including their biotransport (Janetski et al., 2012; Murphy et al., 2012; Gerig et al., 2018), while being a globally important freshwater resource (Bunnell et al., 2014).

BIOLOGICAL TRANSPORT OF CONTAMINANTS

Contaminant biotransport is the dispersal of contaminants by organisms via movement within or among systems. The process of contaminant biotransport is characterized by several steps including: (1) contaminant bioaccumulation by a migratory organism; (2) contaminant transport across an ecosystem boundary; and (3) contaminant deposition into the recipient ecosystem (Blais et al., 2007; Kallenborn and Blais, 2015). This process can include a fourth step related to variables that control how biotransported contaminants are accumulated in the recipient food web (Gerig et al., 2018). Furthermore, life history traits of the biovector including breeding strategy (e.g., semelparity), large size, high trophic position, increased fecundity, and synchronicity of movement enhance the likelihood an organism will biologically transport contaminants (Janetski et al., 2012; Schiesari et al., 2018). Both migratory fish and colonial nesting sea birds are prominent examples of contaminant biovectors due to their contaminant burdens, movement patterns and ability to liberate contaminants into a new ecosystem (Krümmel et al., 2003; Michuelleti et al., 2010). Examples of contaminant biotransport run counter to the more usual unidirectional flow of material, such as with flowing water or eroding soils, or from land to sea, thereby facilitating a connection between areas without contaminants and those areas replete with contaminants. Moreover, biovectors accumulate contaminants over a broad spatial area and focus them into a much smaller area. For example, Pacific salmon introduced to the Great Lakes accumulate contaminants from across an entire lake basin prior to migrating to streams to spawn, thereby focusing their contaminant burden into a small area.

The introduction of Pacific salmon (*Oncorhynchus* spp.) to the Laurentian Great Lakes in the mid-1960s provides a valuable setting to evaluate the role of contaminant biotransport. Pacific salmon were introduced to control alewife (*Alosa pseudoharengus*) and establish a sport fishery (Crawford, 2001; Dettmers et al., 2012). However, establishment of salmon coincided with peak levels of many POPs, which bioaccumulate in aquatic food webs and have negative consequences for fish, wildlife, and human health (Murphy et al., 2012). In the 60 years since salmon were stocked, salmon have become naturalized to many streams and exhibit natural recruitment (Dettmers et al., 2012). As a consequence, many Great Lakes tributaries have annual runs of Pacific salmon that facilitate the translocation of contaminants from lake to tributary (**Figure 1**). Thus, migratory salmon can be considered a source of contaminants to tributaries of the Great Lakes region, now and in the future (Janetski et al., 2012; Gerig et al., 2018).

CONTAMINANT BIOTRANSPORT IN THE LAURENTIAN GREAT LAKES

Research from the Great Lakes over the last decade has increased our understanding of contaminant biotransport by Pacific salmon. We now appreciate that stream-resident fish can acquire contaminants from salmon spawners (Janetski et al., 2012; Gerig et al., 2018). Moreover, the primary mechanism by which salmon facilitate contaminant dispersal and bioaccumulation is through their eggs, which are consumed readily by stream-resident fish (Gerig et al., 2019). Thus, salmon create a direct linkage between the Great Lakes and a vast tributary network facilitating the translocation of contaminants to locations often

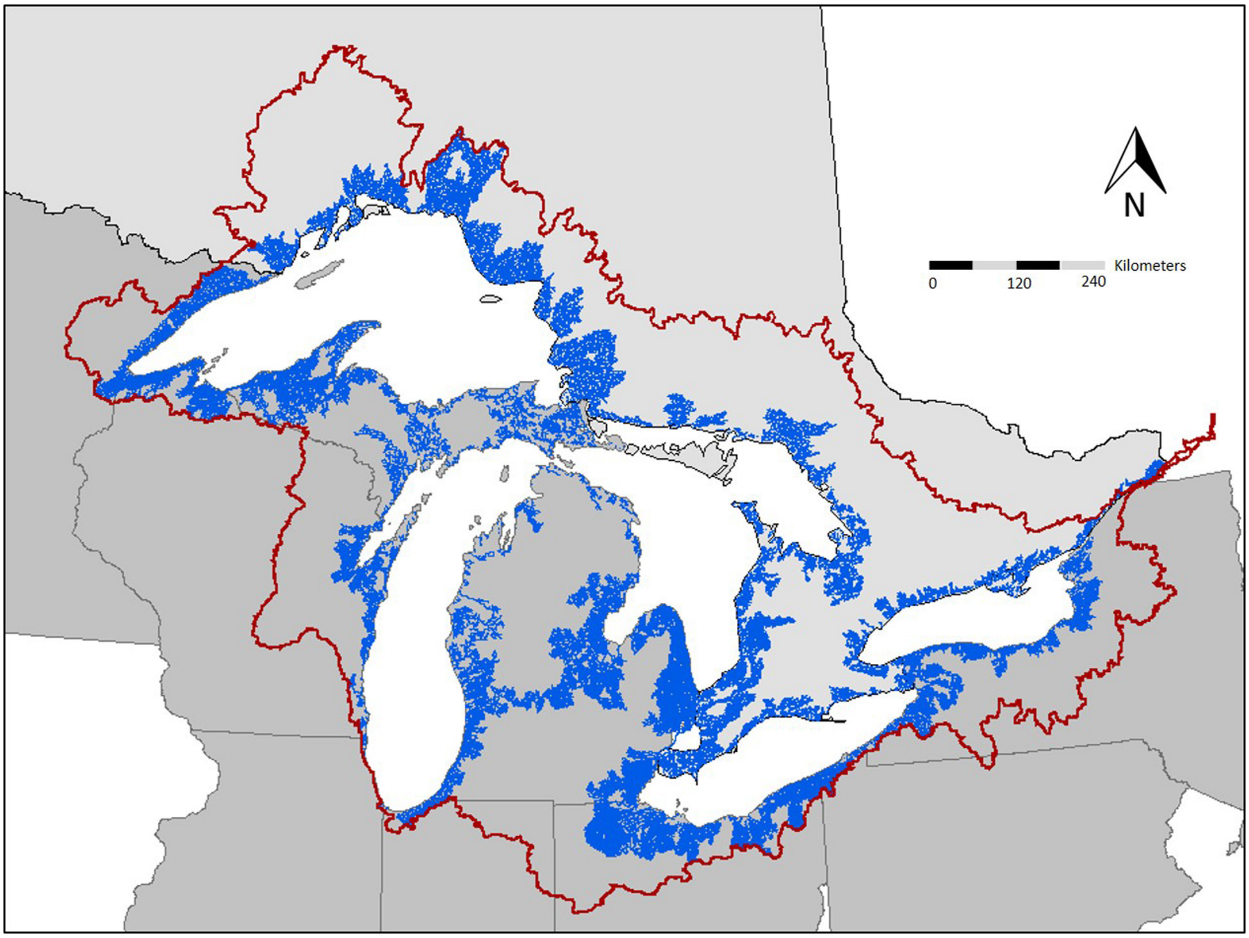

FIGURE 1 | Potential extent of contaminant biotransport in the Great Lakes basin. The dark red line represents the Great Lakes watershed boundary. The blue lines represent tributaries that are accessible to migratory fish. Tributary accessibility data obtained through open source data acquired through Great Lakes aquatic habitat framework.

lacking direct point sources of contamination (Janetski et al., 2012; Gerig et al., 2018).

Prior studies suggest the quantity of a contaminant deposited to a stream is strongly influenced by two factors – the contaminant burden and the abundance of the salmon. That relationship can be summarized in the following equation:

$$\begin{aligned} &\textit{Contaminant flux}\ (ng\ m^{-2}) \\ &\quad = \textit{Mean contaminant load of biovector}\ (ng) \\ &\qquad \times \textit{Abundance per unit habitat area}\ (N\ m^{-2})] \end{aligned}$$

The quantity of contaminants delivered by salmon can increase either as a function of highly contaminated individuals migrating at low densities, less contaminated individuals migrating at high densities (Krümmel et al., 2003), or the interaction of both (Janetski et al., 2012; Gerig et al., 2018). This relationship can be further enhanced or diminished by the habitat area that salmon occupy for spawning.

In the Great Lakes, contaminant biotransport varies among Lakes Michigan, Huron, and Superior with Lake Michigan exhibiting the greatest, Lake Huron exhibiting intermediate, and Lake Superior exhibiting minimal biotransport of PCBs (**Figure 2A**; Janetski et al., 2012; Gerig et al., 2016). This gradient reflects the contaminant levels of spawners and their densities. Contaminant biotransport in Lake Michigan is enhanced by highly contaminated Chinook salmon (*Oncorhynchus tshawytscha*) spawning at high densities, while Lake Superior tributaries receive comparatively small runs of lesser contaminated coho (*Oncorhynchus kisutch*). This pattern suggests that the salmon biotransport of POPs to streams is influenced by species- and basin-specific factors. For instance, Chinook salmon have higher trophic positions, reach larger sizes, and bioaccumulate greater contaminant burdens than coho, making them a more effective biovector (Gerig et al., 2016). In addition, Lake Michigan has (1) a thermal regime and prey fish populations that supports larger salmon biomass, both in terms of overall abundance and fish size, than Lake Superior (Dettmers et al., 2012; Bunnell et al., 2014), and (2) higher levels of current and historic contamination than Lake Superior (Hornbuckle et al., 2006). Salmon biotransport of Hg is also observed in the

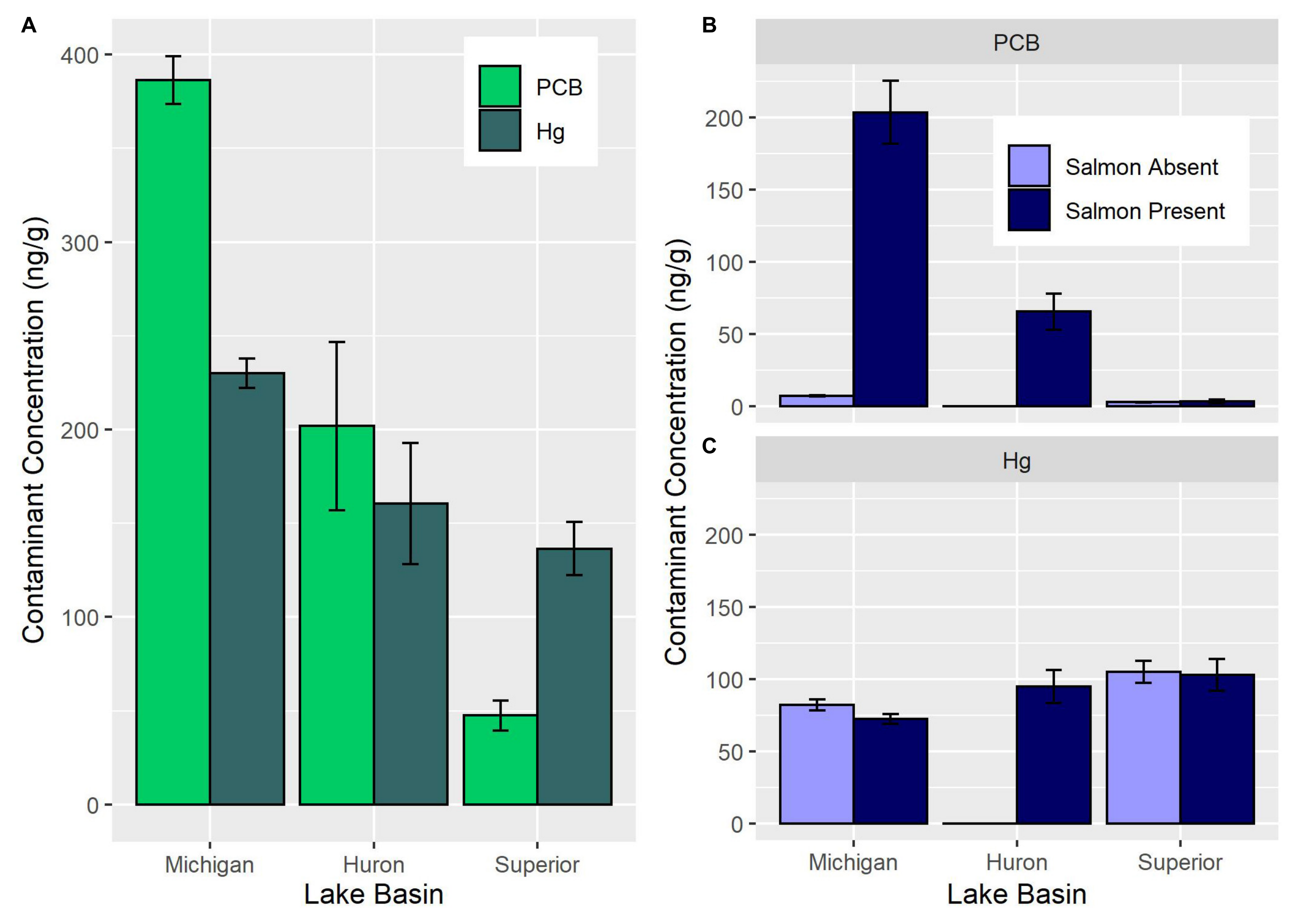

FIGURE 2 | Spawning Pacific salmon exhibit basin-specific differences in PCB and Hg concentrations across Lakes Michigan, Huron, and Superior **(A)**. Stream-resident fish exhibit PCB concentrations that differ among lake basins and between locations open to Pacific salmon **(B)**. Stream-resident fish exhibit Hg concentrations that differ among lake basins but not with the presence of Pacific salmon **(C)**. Note the magnitude of difference is much more pronounced for PCB than Hg. No stream-resident fish were collected from salmon absent Lake Huron tributaries. All concentrations expressed as ng/g wet weight. Data source: Gerig, 2017.

Great Lakes (Sarica et al., 2004; Gerig et al., 2018). The magnitude of difference in salmon Hg concentrations among Upper Great Lakes basin is smaller than POPs due to smaller differences in atmospheric deposition among the Upper Lakes (Risch et al., 2017). However, similar to the POP pattern, Hg concentrations are highest in Lake Michigan and lowest in Lake Superior (Gerig, 2017). Understanding factors controlling the transport of contaminants to tributaries is the first requirement for understanding how salmon alter bioaccumulation in tributaries.

Contaminants accumulate at different rates in fish tissues based upon the biochemical properties (e.g., protein or lipid content) of the tissue (e.g., muscle, gametes). As such, differential bioaccumulation between tissues can be used to determine the trophic pathway responsible for increased resident fish bioaccumulation. Salmon deposit eggs and carcasses in streams during spawning. Salmon eggs have higher PCB concentrations than muscle and very low concentrations of Hg (Gerig et al., 2018). PCBs are lipophilic and accumulate readily into fat-rich tissues, such as fish eggs (Blais et al., 2007; Murphy et al., 2012). In contrast, Hg binds to proteins in muscle tissue (Kuwabara et al., 2007), which is largely absent from eggs and therefore limits Hg accumulation. Thus, consideration of PCB and Hg concentrations together is an effective tool to identify how salmon influence stream-resident fish bioaccumulation.

Bioaccumulation of biotransported contaminants by stream-resident fish is dependent upon whether POPs or Hg are considered. Stream-resident fish including native brook and introduced brown trout exposed to salmon runs exhibit a 24-fold higher PCB concentration than fish from non-salmon reaches (**Figure 2B**; Gerig et al., 2018). Moreover, PCB concentrations in stream-resident fish increase non-linearly with the quantity of PCB delivered by salmon (**Figure 3**; Gerig et al., 2018). Similarly, concentrations of other POPs, like DDE and PBDE, in stream-resident fish also increase with the increased flux of salmon derived contaminants (Janetski et al., 2012). However, DDE and PBDE concentrations in salmon spawners are low compared to PCBs, reflecting lower environmental concentrations (Janetski et al., 2012; Murphy et al., 2012). Together, these findings suggest that other POPs such as Mirex, Dieldrin, and Chlordane with similar properties to PCBs are subject to biotransport

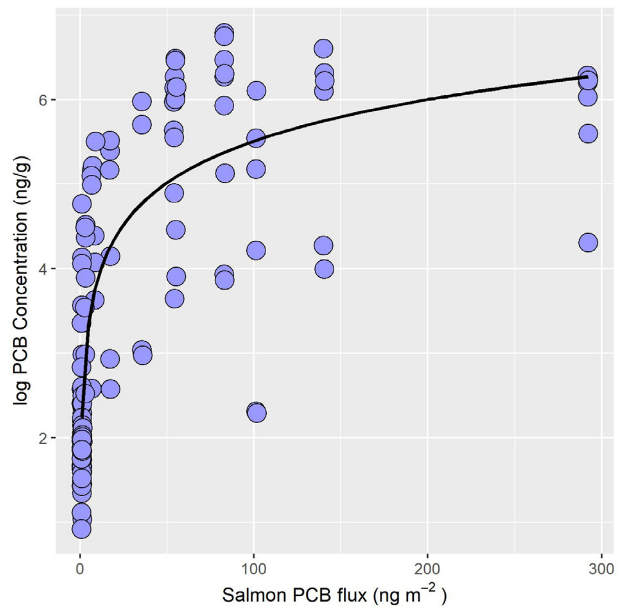

FIGURE 3 | PCB concentrations (ng/g wet weight) of stream-resident fish increase non-linearly with the flux of PCB (ng m^{-2}) supplied by salmon. Salmon PCB flux calculated from visual abundance estimates, biometric data, and mean PCB burden of salmon. Data source: Gerig, 2017.

FIGURE 4 | A native brook trout that had gorged on salmon eggs in a northern Lake Michigan tributary. Photo credit: David Janetski.

by Pacific salmon (e.g., O'Toole et al., 2006). However, the extent of biotransport reflects the relative concentration in the environment.

Mercury is recognized as another important contaminant in the Great Lakes region. Despite evidence of Hg biotransport by salmon (Sarica et al., 2004; Gerig et al., 2018), stream-resident fish in the Great Lakes have reduced or negligible differences in Hg between locations with and without salmon (**Figure 2C**; Gerig et al., 2018). Moreover, Hg in stream-resident fish decline as Hg supplied by salmon and stream-resident fish size increases (Gerig et al., 2018). In contrast, a mesocosm experiment that measured the effect of carcass consumption on Hg bioaccumulation determined that brook and brown trout Hg increased with increased salmon consumption (Gerig et al., 2017). However, similar to field studies from the Great Lakes, Dolly Varden char (*Salvelinus malma*) in Alaska have lower Hg concentrations as the abundance of spawning salmon increases (Cyr et al., 2016). If salmon-derived contaminants were accumulated primarily through carcass consumption, then data from observational studies should demonstrate a paired increase in both PCB and Hg concentrations much like what has been observed in Grizzly Bears (Christensen et al., 2005). These results suggest that understanding the trophic ecology of the receiving food web is essential to understanding how biotransported contaminants are bioaccumulated.

Diet and ration size interact to influence the bioaccumulation of salmon derived contaminants. Stream-resident fish subjected to salmon in the Great Lakes shift their diet to consume energy-dense salmon eggs during fall salmon runs (Ivan et al., 2011; Gerig et al., 2019). Stream-resident fish have been found to gorge on salmon eggs with ration size increasing 14-fold from pre-salmon run levels. When salmon are present, eggs account for ∼70% of their diet (**Figure 4**; Gerig et al., 2019). However, widespread variability in the degree of egg consumption (Gerig et al., 2019), both in space and time, suggests that dietary plasticity among individuals can enhance exposure to POPs. Moreover, differential bioaccumulation of POPs and Hg by stream-resident fish may be modulated by variability in egg consumption. A simulation model parametrized using field data demonstrated a trade-off between PCB and Hg bioaccumulation that was mediated by the degree of egg consumption (Gerig et al., 2019). Increased egg consumption resulted in increased stream-resident fish growth and PCB concentration while leading to a simultaneous decline in Hg concentration attributed both to the low mercury content of eggs and somatic growth dilution (Gerig et al., 2019). Previous research in locations lacking biovectors has found that stream-resident fish PCB and Hg concentrations vary with physical and chemical factors related to water chemistry and land cover (King et al., 2004). However, salmon deliver such a large contaminant flux that other factors that would otherwise modulate stream-resident fish bioaccumulation are overshadowed. The stronger influence of biological over physicochemical factors parallels other research on the ecological effects of salmon spawners where the stable isotope ratio of resident fish was more strongly influenced by the salmon run size than by instream or landscape-level variables (Reisinger et al., 2013; Swain et al., 2014). This highlights the strong influence that salmon have on ecosystems as ecosystem engineers as well as provision of resource subsidies (Schindler et al., 2003).

Salmon release considerable quantities of POPs and Hg through carcass deposition that could influence bioaccumulation in other food web components. A large portion of salmon-derived POPs and Hg is transported downstream following carcass decomposition (Sarica et al., 2004; O'Toole et al., 2006). O'Toole et al. (2006) found that semi-permeable membrane devices accumulated more POPs when spawning and decomposing salmon were present. In addition, Sarica et al. (2004) observed increased aqueous concentrations of total and methylated Hg during carcass decomposition. While localized salmon spawning can increase the aqueous concentration of POPs and Hg, neither study determined whether increased aqueous concentrations correlated with increased bioconcentration or food web bioaccumulation. Sarica et al. (2004) also found a significant increase in Hg concentrations in for aquatic invertebrates from scavenging or collector-gathering feeding guilds. Limited data exists on the effect of salmon on stream invertebrate POP concentrations but we would anticipate invertebrates to respond, similarly, to Sarica et al. (2004). In contrast, the gut microbiome of stream invertebrates from a wide range of feeding guilds did not change as a result of an experimental salmon carcass introduction suggesting minimal use of this resource (Larson et al., 2019). In addition, a previous study noted that black bears (*Ursus americanus*) translocated a significant quantity of Hg to the riparian zone of the Credit River in the Lake Ontario watershed significantly diminishing the quantity of Hg available for downstream transport (Sarica et al., 2004). POPs were not measured in this study, but we would expect for POPs transported to riparian zone by bears. Observations of carcass translocation to the riparian corridor has not been noted in the Upper Great Lakes (Janetski et al., 2012; Gerig et al., 2018) but should be explored in the future.

FUTURE DIRECTIONS AND MANAGEMENT

Past research has focused narrowly on semelparous Pacific salmon when assessing migratory fish contaminant biotransport (Gregory-Eaves et al., 2007; Janetski et al., 2012; Gerig et al., 2018). However, many different migratory fish have the potential to transport contaminants. Previous reviews suggest iteroparous fish, such as steelhead or suckers, represent a diminished risk for contaminant biotransport because they only deposit gametes (Blais et al., 2007; Kallenborn and Blais, 2015). However, eggs represent a POP-rich food resource that fish readily gorge upon when available (Gerig et al., 2019). Different migratory fish have different spawning strategies, such as broadcast vs. nest spawning, which may impact the availability of eggs for consumption. In addition, fish eggs, even within the *Oncorhynchus* genus, exhibit differential provisioning, leading to variability in size and lipid content thereby influencing POP concentrations (Quinn, 2018). Determining what species of migratory fish function as significant biovectors will require a detailed trait-based analysis (cf. Schiesari et al., 2018). For example, in the Great Lakes over 50 fish species have been identified as having a migratory or partially migratory life history (Lane et al., 1996). The fish species associated with Great Lakes tributaries differs markedly with respect to contaminant burden, abundance, fecundity, spawning mode, run timing, and swimming ability with consequences for their individual capacity to accumulate, transport, and deliver contaminants. Identifying variables that enhance or diminish contaminant biotransport is an important step to managing inputs of biotransported contaminants.

Biotransported contaminants present a challenge from a management perspective because they defy the conventional paradigm of pollution flowing from upstream to downstream. Illustrating this challenge, biotransport is seldom included in risk assessments used to construct fish consumption advisories. Based upon an analysis of data presented in Gerig (2017) from the Lake Michigan and Huron Basin, 50% of stream-resident trout from streams open to salmon had PCB concentrations that exceeded the no fish consumption guideline for cancer causing and 25% exceeded non-cancer causing endpoints (**Figure 5**; US Environmental Protection Agency, 2000). This finding points to an opportunity where existing contaminant databases could be leveraged to identify "hot-spots" of biotransport that could subsequently be targeted for consumption advisories and public outreach (Gerig et al., 2018). However, effective use of that information will require better understanding of the biology of contaminant transport, especially in the context of local chemical and physical conditions, which are strongly driven by human activities.

One important factor in the Great Lakes region is the large number of tributary dams that impact ecosystem connectivity. Environmental contaminants (Murphy et al., 2012) interact with aging dam infrastructure (Stanley and Doyle, 2003) to create ecological stressors that impact the fisheries and watersheds. Removal of obsolete dams can improve lotic function by increasing sediment transport, restoring natural thermal and flow regimes, and extending migration corridors for fish

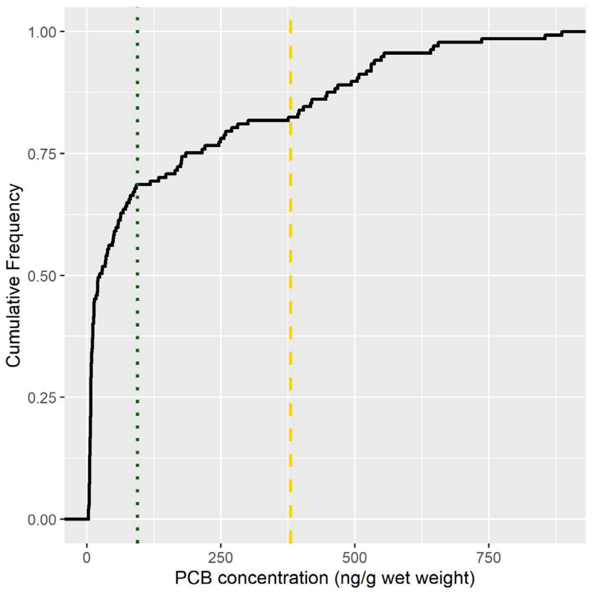

FIGURE 5 | Cumulative proportion of stream-resident trout PCB concentrations (ng/g wet weight) that exceed EPA cancer (dashed line) and non-cancer causing (dotted line) end points. Cancer end points exceed 380 (ng/g wet weight) while non-cancer exceed 94 (ng/g wet weight). Non-cancer health endpoints may include immune, reproductive, neurological, or endocrine impairments. All fish included in this analysis were sampled in streams draining to Lake Michigan or Huron that receive annual salmon runs. Data source: Gerig, 2017.

(Poff et al., 1997). However, dam removal may also increase the likelihood of invasive species colonization and contaminant biotransport by migratory fish (**Figure 1**; McLaughlin et al., 2013; Gerig et al., 2019). Although dam removal can provide ecological benefits, careful consideration and prioritization should be employed to minimize other impacts (Rahel, 2013), including the risk of contaminant transport (Janetski et al., 2012). Future management could involve developing a decision-making process that simultaneously minimizes biotransport and non-native species dispersal while restoring connectivity for native species. For example, managers could identify species likely to have the most impact in terms of contaminant biotransport and establish seasonal barriers that would selectively prevent those species from migrating upstream; seasonal barriers have successfully been implemented in the Great Lakes to limit expansion of Sea Lamprey (*Petromyzon marinus*) into tributaries (Lavis et al., 2003). Selective barriers may be a feasible management option for species that migrate with a predictable phenology if ecosystem and human health impairments are apparent. One key tool in development of that process will be to establish a decision-making framework for use by managers to address costs and benefits of such actions.

Recent modeling efforts have demonstrated the ability to identify mechanisms controlling contaminant biotransport (McGill et al., 2017; Gerig et al., 2019). However, these assessments have been restricted to POPs such as PCBs, which are declining and currently banned from production (Murphy et al., 2012). In contrast, more than 1,000 chemicals have been identified as having the ability to bioaccumulate with one-third exhibiting potentially toxic effects on aquatic biota, wildlife, and human health (Murphy et al., 2012; Walters et al., 2016). Moreover, many thousands of chemicals are developed every year with only minimal consideration of their environmental impact (Walters et al., 2016). Expansion of recent models could be leveraged to determine the risk of biotransport of emerging contaminants that are hydrophobic, resistant to degradation, and present a clear bioaccumulation risk. This effort may be a particularly useful application of models because adequately screening all potential chemicals that pose regional and global risks remains challenging (Walters et al., 2016).

This mini-review synthesizes our current understanding of the mechanisms and variation surrounding the process of contaminant biotransport by Pacific salmon, an issue of high importance given widespread introductions of salmon on multiple continents. Overall, research at differing scales and levels of control has demonstrated that salmon can have a marked impact on stream-resident fish contaminant burdens. However, salmon do not uniformly impact the stream-resident fish community and the magnitude of their effect appears tightly linked to the biological context related to the specific contaminant, species identity, and trophic pathway to contamination, which interact to determine the magnitude of salmon biotransport and uptake. Consequently, consideration of the recipient food web and route of exposure is critical to understanding the fate of biotransported contaminants in ecosystems.

AUTHOR CONTRIBUTIONS

BG, DJ, DC, and GL had equal contributions to the development, writing, and revision of this manuscript. All authors contributed to the article and approved the submitted version.

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A River of Bones: Wildebeest Skeletons Leave a Legacy of Mass Mortality in the Mara River, Kenya

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Animal carcasses can provide important resources for a suite of consumers, and bones may provide a largely overlooked component of this resource, as they contain a large proportion of the phosphorus (P) in a carcass and they can persist for decades to millennia. We synthesized several datasets from our research in the Mara River, in which annual mass drownings of wildebeest (*Connochaetes taurinus*) contribute 2.2×10^5 kg of bones per year, to examine the ecological role that bone could play in this river ecosystem and to prioritize research questions on the role of bones in aquatic ecosystems in general. We measured bone stoichiometry and used in-stream litterbags to measure bone decomposition rate, both of which varied by bone type. Decomposition occurs as a two-stage process, with 15% of the mass being relatively labile and decomposing in 80–120 days and the remaining recalcitrant portion decomposing over > 80 years, leading to an estimated standing stock of 5.1×10^6 kg bones in the river. We used mesocosm experiments to measure leaching rates from bones. Leachate from fresh bones was an order of magnitude higher in inorganic nitrogen (N) than P; however, aged bones from the river leached much more P than N, which stimulated primary production. Biofilms growing on bones had five times greater chlorophyll *a* and two times greater organic matter than those growing on rocks, although algal composition was not significantly different between the two substrates. Biofilms growing on bones also differed from biofilms on rocks in carbon (C) and N stable isotope signature. Mixing models suggest that biofilms on bones account for 19% of macroinvertebrate and 24% of fish tissues in the Mara River, even months after carcasses were present. In combination, these findings suggest that bones may influence nutrient cycling, ecosystem function, and food webs in the Mara River, potentially on decadal time scales. Bones may also be important in other aquatic ecosystems, and mass extirpations of large land mammals may have led to a loss of this resource. Large animal bones may play a unique role in ecosystems via their slow release of limiting nutrients.

Keywords: aquatic ecosystem, bone, carcass, decomposition, production, river, skeleton, stoichiometry

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INTRODUCTION

Animals can have myriad effects on biogeochemistry, nutrient cycling, and ecosystem function through both direct and indirect effects on trophic processes and through transport processes (Bauer and Hoye, 2014; Schmitz et al., 2018). Animals tend to aggregate in time and space, which can lead to biogeochemical hot spots and hot moments (McClain et al., 2003), and animals can move across ecosystem boundaries, which can transport resource-rich subsidies against natural gradients or at an elevated rate along natural gradients (Subalusky and Post, 2018). Live animals can play important roles in driving these dynamics through nutrient assimilation and excretion, during which animals can serve as sinks for some elements by assimilating them in their body tissue (Kitchell et al., 1979; Atkinson et al., 2016; Nobre et al., 2019). After death, animal carcasses may play an important role as a nutrient source by liberating limiting nutrients (Bump et al., 2009; Beasley et al., 2012; Keenan et al., 2018).

Animal carcasses provide a complex and heterogeneous resource for an array of consumers. Animal carcasses can result from annual or seasonal mortality associated with normal life history, selective drivers of mortality (e.g., disease, predation, hunting), or mass mortality events (Wilmers et al., 2003; Ameca y Juárez et al., 2012; Fey et al., 2015; Wenger et al., 2019). The resulting differences in the abundance, location, and timing of carcass deposition, as well as in animal characteristics including body size and stoichiometry, can have pronounced effects on decomposition and utilization of carcass components (Tomberlin and Adler, 1998; Beasley et al., 2012; Subalusky and Post, 2018). Carcass decomposition is a multi-stage process: an early stage of decomposition characterized by high rates of elemental leaching, an active stage characterized by microbial and insect colonization, and an advanced stage characterized by physical/mechanical breakdown and chemical dissolution of bones (Parmenter and Lamarra, 1991; Keenan et al., 2018). The earlier stages can be relatively rapid, occurring over days to months, as compared to the latter stage, as bones can persist for decades to millennia (Vereshchagin, 1974; Smith and Baco, 2003; Miller, 2011; Miller et al., 2013).

Much research has focused on the influence of soft tissues, which provide the majority of carcass resources for invertebrates and small-bodied vertebrates. Soft tissues are high in nitrogen (N) and phosphorus (P), which are often limiting nutrients in ecosystems. The stoichiometric ratio of N to P in soft tissues ranges from 10 to 100:1 (Elser et al., 1996). Soft tissues decompose over days to weeks, but they can have pronounced and rapid ecological effects that can persist for long periods of time (Parmenter and Lamarra, 1991; Chaloner et al., 2002; Regester and Whiles, 2006; Bump et al., 2009; Parmenter and MacMahon, 2009; Pray et al., 2009).

Much less research has focused on the decomposition and utilization of bones from animal carcasses (Wambuguh, 2008; Wenger et al., 2019). Bone is a composite material consisting of a mineral phase, an organic phase, and water (Currey, 2002). The mineral phase is comprised of calcium phosphate primarily in the form of hydroxylapatite. The organic phase is comprised of collagen, non-collagenous proteins, and lipids. As a result of this structure, bones have a much higher proportion of P than soft tissues, with N:P ratios of $< 1:1$ (Elser et al., 1996; Subalusky et al., 2017), and bones can persist in ecosystems for decadal time scales (Smith and Baco, 2003; Wenger et al., 2019). Because the scaling of bone and body size in terrestrial vertebrates is non-linear, larger-bodied vertebrates have a much larger proportion of their total body mass comprised of bone than small-bodied animals (Prange et al., 1979; Elser et al., 1996). Altogether, these studies suggest bones may provide a long-term, P-rich resource, particularly when bones result from carcasses of large vertebrates, raising questions about the role they may play in nutrient cycling and consumer dynamics.

The fate of bones in an ecosystem is largely influenced by environmental context. Bones in terrestrial ecosystems are subject to decomposition via exposure to sun and rain, fungus, and foraging by animals that can consume bones, including rodents and larger animals such as hyenas. Bone persistence in tropical, terrestrial ecosystems is on the scale of several decades (Behrensmeyer, 1978; Trueman et al., 2004; Western and Behrensmeyer, 2009; Ross and Cunningham, 2011). Bone decomposition in temperate and arctic latitudes, which are cooler and drier, can extend over millennial time scales (Vereshchagin, 1974; Andrews, 1995; Wambuguh, 2008; Miller, 2011; Michelutti et al., 2013). In marine ecosystems, the limited role of bacteria and the temporal stability of environmental conditions can result in slow decomposition rates that foster the development of specialist assemblages on carcasses (Baco and Smith, 2003; Smith and Baco, 2003; Beasley et al., 2012). Bone decomposition in aquatic ecosystems may be slowed by occasional burial in benthic sediments (Johnston et al., 2004). Studies suggest only 10–15% of fish bone may be lost due to permanent burial (Vallentyne, 1960; Schenau and De Lange, 2000; Vanni et al., 2013), although this rate likely varies widely depending on characteristics of the aquatic ecosystem and has not been well-studied. Despite these burial rates, accumulation of detritus from fish bones in marine benthic sediments can comprise a significant portion of sediment P and lead to high rates of phosphate fluxes under certain conditions (Schenau and De Lange, 2001).

Aquatic ecosystems likely have higher densities of bones than terrestrial ecosystems because, in addition to mortality of aquatic vertebrates, they may also be a source of mortality for terrestrial animals as well as aggregate slowly-decomposing bones from the terrestrial landscape (Behrensmeyer, 1982; Wenger et al., 2019). There is a long history of studying the origin and persistence of bonebeds and fluvial transport of bones in paleoecology (Behrensmeyer, 1988, 2007). However, little work has focused on the potential ecological effects of bones in aquatic ecosystems. The disparity in the amount of ecological research on carcasses versus that on bones is illustrated in a Web of Science search conducted on 14 March 2019 for literature on the topic. Studies on carcass decomposition in aquatic ecosystems [(carcass OR body) AND (decomposition OR decay) AND aquatic] since 1990 yielded 218 studies, as compared to a search for studies on bone decomposition [(bone OR skeleton) AND (decomposition OR decay) AND aquatic] that yielded only 25 studies (**Figure 1**).

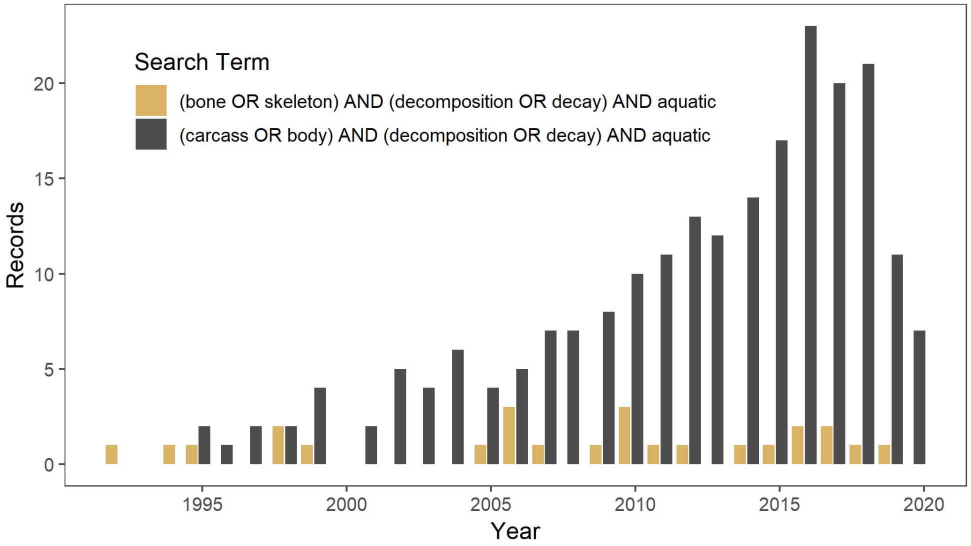

FIGURE 1 | Web of Science search conducted on 14 March 2019 for literature since 1990 on decomposition in aquatic ecosystems of carcasses (in gray) versus bones (in brown).

The Serengeti wildebeest (*Connochaetes taurinus*) migration provides an opportunity to examine the influence of large inputs of bones from large mammals on river ecosystem function, and raises interesting questions about the ontogeny and effects of animal bones in aquatic ecosystems (**Figure 2**). Annual mass drownings in the Mara River result in the input of an average of 6,250 carcasses into the river every year (Subalusky et al., 2017). Approximately half of a wildebeest carcass is soft tissue, which decomposes over weeks to months, but the other half is bone, which comprises 95% of the phosphorus (P) in a carcass and decomposes over years (Subalusky et al., 2017). The pulsed input of these carcasses influences nutrient cycling in the river on annual time scales (Subalusky et al., 2017, 2018), but there may also be long-term effects on nutrient cycling and river food web dynamics through the persistence of bones.

Here we synthesize several datasets from our research in the Mara River to examine the ecological effects that bone could have on nutrient cycling, ecosystem function, and food web structure in the river. We use these data and our preliminary understanding of the role of bones in this ecosystem to propose several research questions to improve our broader understanding of the role of mammal bones in aquatic ecosystems. We also suggest this may be an overlooked phenomenon in other rivers and may have been particularly important in the past when robust populations of large mammals were more common.

MATERIALS AND METHODS

Study Site

This research took place in the Mara River, which runs through the Maasai Mara National Reserve in Kenya and the Serengeti National Park in Tanzania. The river hosts a population of > 4,000 hippopotamus (*Hippopotamus amphibius*) and the annual migration of 1.3 million wildebeest, which both provide important resource subsidies to the river (Subalusky et al., 2015, 2017). The Serengeti wildebeest migration is in the Kenyan portion of the Mara River basin from July to November, and the animals cross the Mara River multiple times during this period as they move between dry season feeding grounds. We have documented nearly annual mass drownings of wildebeest during river crossings upstream of the New Mara Bridge near the border between Kenya and Tanzania (Subalusky et al., 2017).

From 2011 to 2015, we estimated a mean of 6,250 wildebeest drowned in the river each year, which contributed approximately 219,200 kg of bones (wet weight) per year to the river (Subalusky et al., 2017). All but one of these drownings occurred within a 5 km reach of river, and carcasses tend to accumulate on river bends and rock outcroppings within a 5 km reach downstream of the drowning location. Thus, if we assume these bones are distributed along a 10 km reach, and the average river width is 40 m, these annual inputs would yield an areal density of 0.55 kg bone/m^2. This estimate does not account for the continual accrual of bones that occurs due to their slow decomposition, and it does not account for the transport of bones farther downstream that likely occurs over time.

All data presented in this paper were from samples collected just upstream of the New Mara Bridge, which is ~200 m upstream of the Tanzanian border, or from an artificial stream experiment that was conducted inside the Maasai Mara National Reserve. All wildebeest bones were collected from the carcasses of animals that drowned naturally in the river. Fishes were sampled using standard field methods. This study was carried out in accordance with the Yale University Institutional Animal Care and Use Committee Animal Use Protocol #2012-10734.

FIGURE 2 | The ontogeny of wildebeest bones in the Mara River, Kenya. **(A)** Annual mass drownings result in the input of a mean of 6,250 carcasses per year. **(B)** Carcass soft tissue decomposes over weeks to months, but bone persists in the river for decades. **(C)** Bones can continue to leach out phosphorus even after a prolonged period in the river. **(D)** Biofilms that grow on bones are higher in chl *a* and organic matter (OM) than biofilms on rocks, and they provide an important food source for macroinvertebrates and fishes.

Bone Decomposition

We measured bone decomposition using three different approaches: (1) measuring *in situ* mass loss of bones in litterbags in the river, (2) measuring changes in the elemental composition of bones after an extended time in the river, and (3) measuring nutrient leaching rates from bones in microcosms.

First, we placed samples of four different types of fresh bone (triplicate samples of leg, rib, scapula, vertebrae; $n = 12$) inside fine mesh ($<500\ \mu$m) litterbags that were secured inside a metal cage in the river. We measured wet mass at five time intervals (between days 0 and 216), and we replaced the same bone samples in the bags after weighing. We did not destructively sample bone for dry mass because of considerable heterogeneity both within and across bone types and difficulty in obtaining a homogenous sample. We calculated decay rate in the R package litterfitter (Cornwell and Weedon, 2014; Cornwell et al., 2014), which allowed us to use AIC model selection to compare a single-pool exponential decay model (Eq. 1), which assumes a homogenous sample with a single decay rate, with a two-pool exponential decay model (Eq. 2), which fits initial mass distributions and parallel decay rates for a sample composed of two different components (e.g., labile and recalcitrant) (Manzoni et al., 2012; Cornwell and Weedon, 2014).

$$\frac{M_t}{M_0} = e^{-kt} \tag{1}$$

$$\frac{M_t}{M_0} = \propto e^{-k_1 t} + (1- \propto)e^{-k_2 t} \tag{2}$$

In both equations, M_0 is the mass remaining at time 0, M_t is the mass remaining at time t, and t is time to decomposition in days. In Eq. 1, k is the constant decomposition rate of the material. In Eq. 2, $\propto$ is the proportion of labile material, k_1 is the decomposition rate of labile material, and k_2 is the decomposition rate of recalcitrant material. All models were run for 500 iterations. We used the resulting parameter values for the selected model to estimate time to 95% mass loss as $\ln(0.05)/k$ for the labile and recalcitrant components.

We also used these parameter values to calculate the steady-state standing stock of bones in the Mara River, according to the following equation:

$$\mathrm{SS} = \frac{\mathrm{I} \propto}{\mathrm{k}_1} + \frac{\mathrm{I}(1- \propto)}{\mathrm{k}_2} \tag{3}$$

In this equation, I is the annual input of bones scaled to a daily rate (600.5 kg wet mass day^{-1}), and $\propto$, k_1, *and* k_2 are from Eq. 2 (Cornwell and Weedon, 2014).

Second, we compared the carbon (C), nitrogen (N), and phosphorus (P) composition of bone samples that were collected fresh (triplicate samples of rib, vertebrae, and joint bones; $n = 9$) with those collected after 216 days of litterbag deployment in the river (triplicate samples of leg, rib, scapula, vertebrae; $n = 12$). Bone samples were dried at 72°C (to meet USDA permit import regulations), lightly sanded to remove any connective tissue from the surface, and finely ground using a cryogenic ball mill. C and N composition were measured using a Costech Elemental Analyzer (Costech Analytical Technologies, Inc., Valencia, CA, United States). P composition was measured by digesting pre-weighed, combusted material using 1M HCl at 80°C for 2 h, treating with an ammonium molybdate color reagent, and analyzing on a flow analyzer (Astoria-Pacific, Clackamas, OR, United States). The percent organic matter (OM) was measured by weighing samples before and after combustion. We used *t*-tests to compare the % OM, % C, % N, and % P in fresh bones versus aged bones of different types (R Core Team, 2018). We compared fresh rib, vertebrae, and joint bones to aged rib, vertebrae, and leg bones, respectively.

Third, we measured initial leaching rates of bone by placing sub-samples of fresh bone (66–98 g, mean = 80 g) in chambers (n = 3) that were filled with 4 L of unfiltered river water and open to the environment. We collected 50 mL water samples for analysis of inorganic nutrients every ∼6 days for 31 days. Water samples were collected using a syringe and filtered through a 0.2 μm Supor polysulfone filter (Pall Corporation, Port Washington, NY, United States) into a sample bottle and frozen until analysis. Samples were analyzed on a portable flow injection analyzer in the field. Ammonium was analyzed using the gas exchange method (APHA, 2006). Nitrate was analyzed using zinc reduction (Ellis et al., 2011). Soluble reactive phosphate (SRP) was analyzed using the molybdate blue method (APHA, 2006). Nutrient concentrations were multiplied by the volume of water in the chamber at each sampling time point to obtain total mass of nutrients leached. We did not correct for background nutrient concentrations in the water we used to fill the chambers, as we did not maintain control chambers over time, but concentrations were very low compared to leaching rates of ammonium and SRP. The mass of the bone sample was multiplied by the % N and % P measured for fresh joint bones, and the ammonium and SRP that leached out over 31 days was measured as a proportion of the total N and P in the bone sample.

Effects of Bones on Ecosystem Function in Experimental Streams

As part of a larger mesocosm experiment examining the influence of wildlife subsidies on ecosystem function, we used recirculating experimental streams to compare the influence of bone versus rock substrates on water column nutrient concentrations, and water column and benthic production. Details of the experimental stream array are in Subalusky et al. (2018). In this experiment, we had 18 individual streams (three blocks of six streams each), and treatments were randomly assigned among each block. The full experiment included controls (n = 4), and four different treatments (n = 2–4). We only present here data from the control streams (n = 4) and the bone treatment streams (n = 2).

One 5-L bucket of washed gravel was placed along the bottom of each stream channel as substrate, and five ceramic tiles were placed in the channel bed for sampling. Streams were filled with 60 L water from Emarti Bridge, which is on the Mara River upstream of the influence of large wildlife, and inoculated with periphyton scrubbed from rocks from New Mara Bridge, within the range of wildlife. Streams were allowed to equilibrate for 1 week, after which treatments were applied, and the experiment was run for two additional weeks. In the bone treatment streams, half of the volume of gravel was replaced with wildebeest bones of unknown age that had been removed from the river. This treatment had approximately 0.7 kg bones m^{-2}, which is comparable to our estimates of areal density of bones in the Mara River.

We used a Manta2 sonde (Eureka Environmental, Austin, TX, United States) containing a Cyclops-7F submersible fluorometer (Turner Designs, San Jose, CA, United States) to measure water column chlorophyll *a* (chl *a*) weekly. We collected water samples weekly to analyze inorganic nutrients, as described above. We also destructively sampled one ceramic tile each week to measure OM of the biofilm as ash free dry mass (AFDM). We filtered a known volume of sample through a pre-weighed, pre-combusted Whatman GF/F filter (GE Healthcare Bio-Sciences, Pittsburgh, PA, United States), and measured AFDM by drying the filter at 60°C, re-weighing it, combusting it for 4 h at 450°C and then re-weighing it to determine mass loss upon combustion. In the final week of the experiment, we scrubbed the biofilm off one ceramic tile from each stream and measured the concentration of chl *a* in a known volume of water using the Manta2 sonde, which we then converted to chl *a* per unit area of tile. *In situ* chl *a* fluorescence can be used as a proxy for chl *a* concentration, although it may provide an overestimate, and direct comparison with chl *a* concentrations requires calibration (Holm-Hansen et al., 2000; Roesler et al., 2017). However, in this analysis, we only compared *in situ* fluorescence values across treatments.

Data were analyzed for normality using a Shapiro–Wilk's normality test in R Core Team (2018), and water column nutrients, water column chl *a*, and benthic AFDM were log-transformed to meet statistical assumptions. We examined differences in water column nutrients, water column chl *a*, and benthic AFDM throughout the duration of the experiment using a linear mixed-effect model run with the lme function in the nlme package in Pinheiro et al. (2016) and R Core Team (2018). We fitted lme models with the restricted maximum likelihood method and a continuous autoregressive temporal correlation structure with week as the repeated factor. Treatment (control, bone) and time (each of 3 weeks of measurement) were treated as fixed effects, and individual streams were treated as random effects. We then used the lsmeans package to perform a Tukey pairwise comparison test between treatments for parameters over the duration of the experiment (Lenth, 2016; R Core Team, 2018). We also analyzed the effect of treatment on biofilm chl *a* at the end of the experiment with a one-way ANOVA using the aov function in R Core Team (2018).

Bone Biofilm

We analyzed chl *a* and OM (measured as AFDM) of biofilms collected from both wildebeest bones and rocks in the Mara River in November 2013 (during the wet season) and February 2014 (during the dry season). At both sampling times, we selected three rocks and three bones from the same reach of river, scrubbed the entire upper surface of the substrate clean using a toothbrush, and analyzed photos of the substrates using ImageJ software to measure the surface area (Schneider et al., 2012). We filtered a known volume of sample through a pre-weighed, pre-combusted Whatman GF/F filter, and measured AFDM as described above. We filtered a known volume of sample through a second Whatman GF/F filter for analysis of chl *a*. We froze the filter paper for > 24 h, extracted the chl *a* using methanol with a basic pH (Holm-Hansen, 1978), and analyzed the samples on a Turner Aquafluor handheld fluorometer (Turner Designs, San Jose, CA, United States). We calculated both chl *a* and AFDM per unit surface area of the substrate. Data were tested for normality using a Shapiro–Wilk's normality test in R, and we analyzed the effect of substrate and season on both parameters using a two-way ANOVA with the aov function followed by a Tukey HSD test in R Core Team (2018).

We analyzed community composition of biofilms from both wildebeest bones and rocks collected from the Mara River in October 2017 and November 2018. In 2017, we scrubbed biofilms from the surfaces of three bones and three rocks randomly selected from the same reach of river, although sampling was not done quantitatively. Samples were preserved with Lugol's solution and counted in the lab at 400x on a Leica DM LS2 compound microscope until 100 algal cells had been reached, and abundance of each taxa was given as a proportion of the total. In 2018, we collected three bones and three rocks from the same reach of river, making sure to collect paired samples from similar depths, and scrubbed 16 cm^2 of surface area. Samples were again preserved with Lugol's solution and counted in the lab. We counted 10 microscope fields for each sample, and we calculated the total abundance of each taxa. We identified both bone and rock periphyton to phylum (Chlorophyta, Chrysophyta, Cyanobacteria, Euglenozoa) (Prescott, 1978), and we parsed Chlorophyta into three functional groups based on growth form (unicellular, colonial, and filamentous). We conducted an analysis of similarity on the community data separately for each year using the anosim function in the vegan package in R Core Team (2018) and Oksanen et al. (2019). The function vegdist is used to create a Bray dissimilarity matrix, and anosim uses the rank order of dissimilarity values to test for statistically significant differences between communities.

Stable Isotopes

We used C and N stable isotopes to examine isotopic differences between biofilms on rocks and biofilms on bones over three different seasons. We collected biofilms from rocks and bones in November 2013 (wet season), February 2014 (dry season), and July 2016 (wet season) ($n = 3$ of each type in each season). We also analyzed the stable isotope signature of fresh wildebeest bones ($n = 8$) collected in 2012–2013 to help interpret differences in biofilm signature between bones and rocks. We then used C and N stable isotopes to partition the contribution of various basal food web resources to the tissue assimilation of aquatic macroinvertebrates and fishes. We used sample data from February 2014, as this was 4 months after any fresh wildebeest carcasses were in the river. This time period should exceed the typical isotope turnover rate for consumer muscle tissue (Vander Zanden et al., 2015) and thus minimize the signal of wildebeest carcass soft tissue in the consumers. We used biofilms growing on rocks and on bones to characterize autochthonous basal food web resources, and we collected samples of hippo feces ($n = 9$), which is the primary source of allochthonous food web resources in this region of the river (Masese et al., 2015; Subalusky et al., 2015, 2018).

We collected 16–30 individuals from each of four families of aquatic macroinvertebrates, including Baetidae, Hydropsychidae, Caenidae, and Simulidae, and we combined individuals into a single bulk sample per family. For Baetidae and Simulidae, we had sufficient individuals to run two replicate samples of 30 individuals each, and we used the mean of those replicates for the stable isotope signatures of those taxa. We also collected tissue samples from the lateral muscle of three species of fishes, including *Labeo victorianus* ($n = 8$), *Labeobarbus altianalis* ($n = 5$), and *Bagrus docmac* ($n = 3$). All samples were collected from near the New Mara Bridge. All samples were dried, ground into a fine powder, and analyzed for $\delta^{13}C$ and $\delta^{15}N$ on a ThermoFinnigan Delta Plus Advantage stable-isotope mass spectrometer (Thermo Scientific, Boca Raton, FL, United States) coupled to a Costech ECS 4010 Elemental Analyzer (Costech Analytical Technologies, Inc., Valencia, CA, United States).

We used Bayesian mixing models in MixSIAR to estimate the proportion of each basal resource assimilated in macroinvertebrate and fish tissue (Moore and Semmens, 2008; Stock and Semmens, 2013). The results of fish assimilation were analyzed and presented by species in Subalusky et al. (2017); here, we analyzed assimilation across aquatic macroinvertebrates and fishes as composite consumer groups. All fish species were omnivorous, so we used 0.4 ± 1.3 for $\delta^{13}C$ (Post, 2002) and 4.3 ± 1.5 for $\delta^{15}N$ (Bunn et al., 2013) for fish trophic enrichment factors, which incorporates variability in trophic structure. We used 0.4 ± 1.3 for $\delta^{13}C$ (Post, 2002) and 1.4 ± 1.4 for $\delta^{15}N$ (Bunn et al., 2013) for macroinvertebrate trophic enrichment factors. We ran models with the normal MCMC parameters (100,000 chain length, 50,000 burn-in). Visual analysis of isospace plots confirmed that consumer data were within the minimum convex polygon of source data, suggesting we were not missing any major diet sources (Phillips et al., 2014).

RESULTS

Bone Decomposition

The *in situ* decomposition of bone in the Mara River was much better described by the two-pool model of decomposition than by the single-pool model for all four bone types (**Table 1**). Results from this model suggest different bone types vary in their decomposition rate. Bones are comprised of 7–27% labile material that decomposes over 78–119 days, and 73–93% refractory material that decomposes over > 80 years (**Table 1**). The k value for the refractory material in all bone types reached the minimum bounds in this analysis package (0.0001), providing a minimum estimate for the time to 95% loss; however, extrapolation beyond this time point is well outside the bounds of what we can infer with the relatively limited duration of our field data (216 days). Scapula and leg bones had the lowest proportion of labile material and as a result decomposed the most slowly (**Figure 3**). Vertebrae bones had the highest proportion of labile material and decomposed more quickly than the other bone types. It is unlikely that bone mass loss during this experiment was due to downstream transport of particulate material, due to the fine mesh size of the litterbags used. Based on an annual

TABLE 1 | Comparison of single- and dual-pool negative exponential models of decomposition for four different wildebeest bone types, parameter values for the best fit model (AIC values in bold; the dual-pool model was the best fit for all bone types), estimates of time to 95% loss for labile and recalcitrant material, and overall% organic matter (OM) and C:N:P stoichiometry by percent mass for wildebeest bones after 216 days in the river and a bison skull after 1000s of years underwater.

	AIC		Labile			Recalcitrant			Standing stock ($\times 10^6$ kg)	Fresh % OM	Aged % OM	Fresh C:N:P	Aged C:N:P
Bone type	**Single-pool model**	**Dual-pool model**	**Proportion**	**k_1 (day^{-1})**	**Time to 95% loss (days)**	**Proportion**	**k_2 (day^{-1})**	**Time to 95% loss (years)**					
Scapula	−14.26	**−38.13**	0.0747	0.0271	111	0.9253	0.0001	>82	5.6		33		14:4:11
Leg*	−21.83	**−36.14**	0.0890	0.0382	78	0.911	0.0001	>82	5.5	48	28	25:4:10	16:3:12
Rib	−17.99	**−34.04**	0.1685	0.0275	109	0.8315	0.0001	>82	5.0	37	27	18:5:11	15:4:11
Vertebrae	−14.68	**−39.75**	0.2672	0.0251	119	0.7328	0.0001	>82	4.4	41	54	23:5:9	38:3:8
Mean (SD)										42 (11)	37 (16)	22:4:10 (4,1,1)	23:4:10 (13,1,2)
Bison**											37		14:4:11

*Fresh joint bone was compared to aged leg bone.**Data from a 3360 year BP (^{14}C age) bison skull found in Clear Lake, IA, United States.*

input of 219,200 kg (wet mass), we estimate the steady-state standing stock of bones in the Mara River is 4.4×10^6 to 5.6×10^6 kg. This estimate is likely high, as it assumes the system is in equilibrium, and it is based on a conservative decay rate due to microbial decomposition that does not account for loss from animal consumption or mechanical breakdown.

There were relatively small differences in the stoichiometry of fresh versus aged bones (those that had been in the river for 216 days) (**Figure 4** and **Table 1**). The mean % OM decreased on average from 42 ± 11% in fresh bones to 37 ± 16% in aged bones. The average stoichiometry of fresh bones (joint, rib, and vertebrae) was 22.1 C: 4.5 N: 9.9 P by % mass compared to 23.1 C: 3.5 N: 10.2 P for aged bones. In leg and rib bones, the % C and % N declined, while the % P increased, likely due to the relatively higher % C and N of labile material in bone (e.g., lipids) and the higher % P of refractory material (e.g., apatite). However, in vertebrae, % C increased as % N and % P decreased over time, which may reflect a greater degree of vascularization and greater initial proportion of labile N and P in this bone type. The only significant changes were the decrease in % N in rib bones (t-test, $t = -6.5_{3.2}$, $p = 0.006$) and the increase in % P in leg bones ($t = -4.25_{3.9}$, $p = 0.014$) (**Figure 4**).

In the chamber experiment, approximately 50% of the mass of SRP and ammonium that leached out of the bones over a month was available after only 3 days (**Figure 5**). The mass of ammonium that leached out after 1 month (96.8 ± 35.7 mg, mean ± SD) was almost an order of magnitude larger than that of SRP (12.7 ± 2.9 mg). Background values from the water used in the chambers (SRP = 0.16 mg, NH_4 = 0.50 mg) were ~1% of the final values. Ammonium appeared to stabilize during the latter half of the month, which may have been due to equilibration with the atmosphere, while SRP continued to increase. A large amount of nitrate available on day 1 (5.0 ± 0.3 mg) was due to the water used in the chamber, which had a background nitrate value of 4.8 mg, but nitrate levels fell to nearly zero by day 3 and stayed there for the duration of the study. This decline was likely due to loss through denitrification due to anoxic conditions in the mesocosms, which we did not measure. We also did not measure other forms of nutrient uptake that may have occurred in these chambers; thus, our estimates of leaching rates are likely conservative. After 31 days, we estimate the bone samples leached out 3.2 ± 0.7% (mean ± SD) of the initial N as ammonium and 0.2 ± 0.0% of the initial P as SRP.

Effects of Bones on Ecosystem Function in Experimental Streams

There was a significant effect of both treatment (LME ANOVA: $F_{5,1} = 213.621$, $p < 0.001$) and time ($F_{5,2} = 22.547$, $p < 0.001$), and a significant interaction between them ($F_{5,2} = 81.530$, $p < 0.001$), on water column SRP in the experimental streams (**Figure 6A**). There was no difference between the bone treatment and the control in week 1, before treatments were applied, indicating similar background conditions. After the treatments were applied, the bone treatment had > 300 times higher SRP than the control treatment in week 2 ($p < 0.001$) and 150 times higher in week 3 ($p = 0.001$) of the experiment.

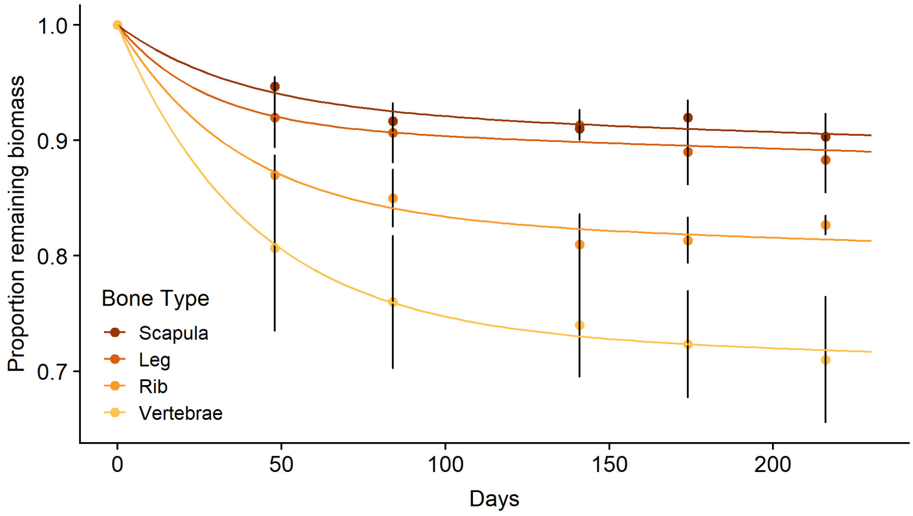

FIGURE 3 | Mean (± SE) proportion of biomass remaining for scapula, leg, rib, and vertebrae bones (n = 3 per bone type) from a wildebeest carcass during litterbag deployment in the Mara River with best fit models following a parallel discrete model of decomposition.

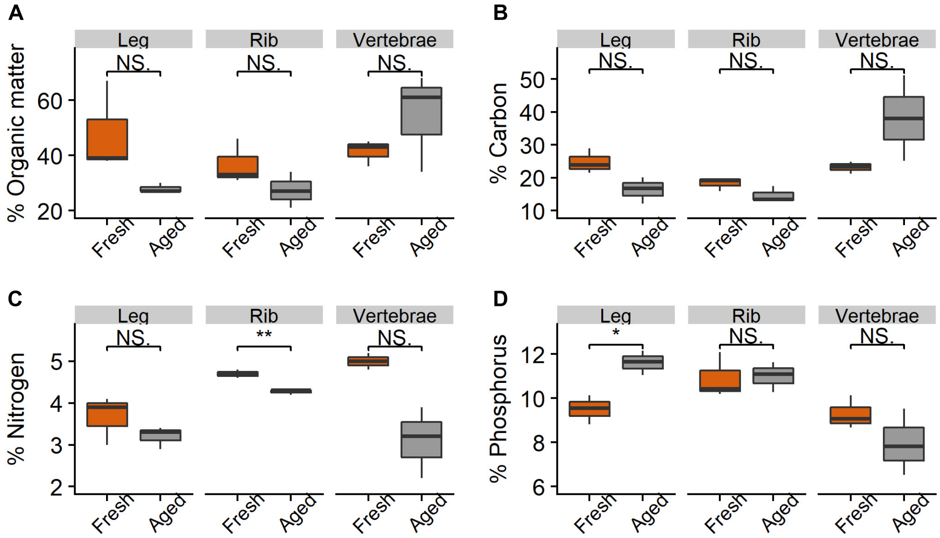

FIGURE 4 | Percent **(A)** organic matter, **(B)** carbon, **(C)** nitrogen, and **(D)** phosphorus in leg, rib, and vertebrae bones (n = 3 per bone type) from wildebeest when fresh and after 216 days in the Mara River. Fresh joint bone was compared to aged leg bone. Asterisks indicate significant difference, where $^{*}p < 0.05$ and $^{**}p < 0.01$.

There was no significant effect of treatment or time, or significant interactions between them, for ammonium (**Figure 6B**). There was a significant effect of time on NO_3 ($F_{5,2}$ = 204.075, $p < 0.001$) although no treatment effect, as both the bone and control treatment declined from ~600 μg L^{-1} NO_3 to nearly zero between weeks 1 and 2 (**Figure 6C**).

There was no significant effect of treatment on water column chl a in the experimental streams, but there was a significant effect of time (LME ANOVA: $F_{5,2}$ = 9.972, p = 0.007) and a significant interaction between treatment and time ($F_{5,2}$ = 17.369, p = 0.001). Chl a was 5 times higher in the bone treatment (364 ± 32) than the control treatment 70 ± 17) in week 2, although this was not statistically significant (p = 0.0737), likely due to low replication (**Figure 7A**). There was no difference between the bone and control treatment in week 1 or week 3.

There was no significant effect of treatment or time, or a significant interaction between them, on tile biofilm AFDM (**Figure 7B**). There also was no significant effect of treatment on tile biofilm chl a at the end of the artificial stream experiment (ANOVA: $F_{5,1}$ = 0.171, p = 0.7).

Bone Biofilm

Biofilm on bones had significantly higher chl a (two-way ANOVA; $F_{1,11}$ = 14.64, p = 0.005) and OM (two-way ANOVA;

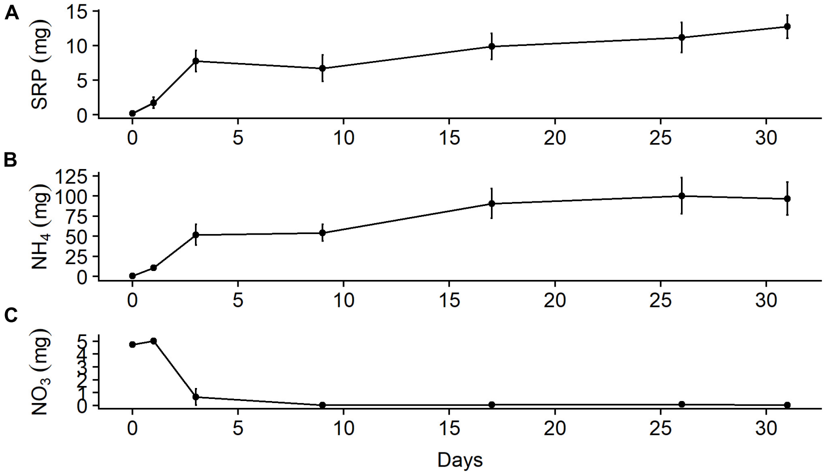

FIGURE 5 | Mean (± SE) total mass in 5-L microcosms (n = 3) filled with river water of **(A)** soluble reactive phosphorus, **(B)** ammonium, and **(C)** nitrate leached out of wildebeest leg bone.

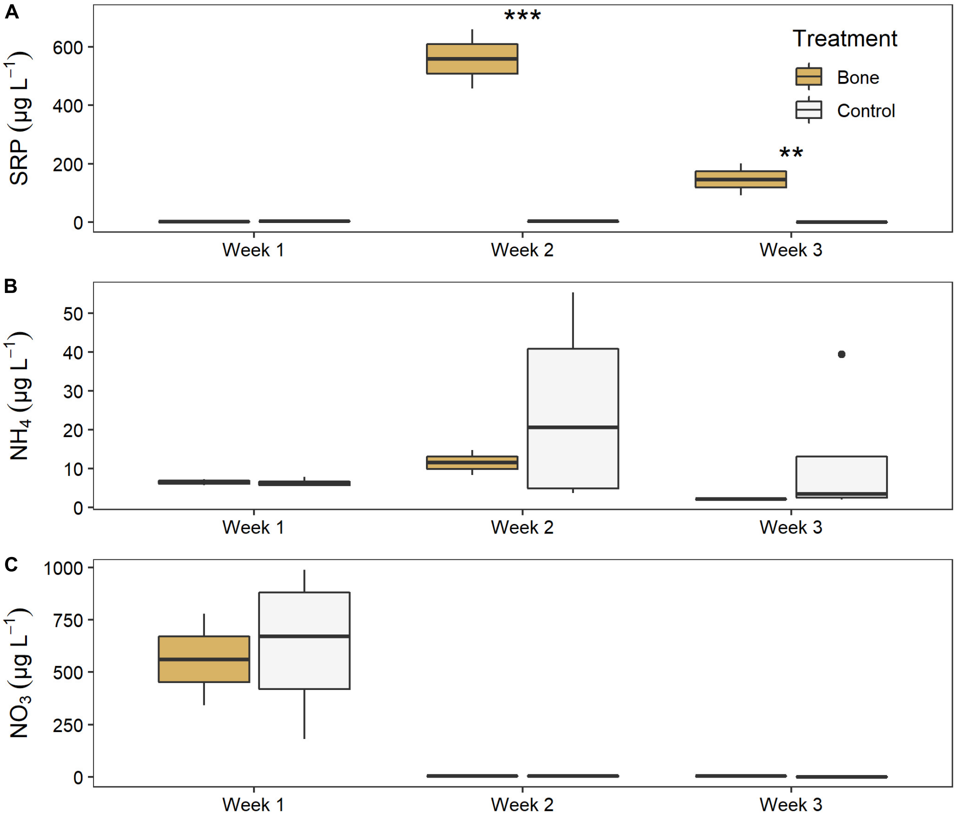

FIGURE 6 | Water column **(A)** soluble reactive phosphorus, **(B)** ammonium, and **(C)** nitrate in experimental streams with all gravel benthos (control treatment; n = 4) or half gravel-half bone benthos (bone treatment; n = 2) in Week 1, after equilibration and just before treatments were applied, and in Weeks 2 and 3 of the experiment. Asterisks indicate significant difference, where $^{**}p < 0.01$ and $^{***}p < 0.001$.

$F_{1,11}$ = 9.13, p = 0.017) than biofilms on rocks (**Figure 8**). Chl *a* was 4.6 times higher and AFDM was 2.0 higher on bone biofilm than on rock biofilm. There was no significant effect of season or interaction between season and substrate.

There was no significant difference between the algal communities in biofilms growing on bones and those growing on rocks in either year (2017: ANOSIM $R = -0.1481$, p = 0.9; 2018: ANOSIM $R = -0.1852$, p = 0.8). The negative R values

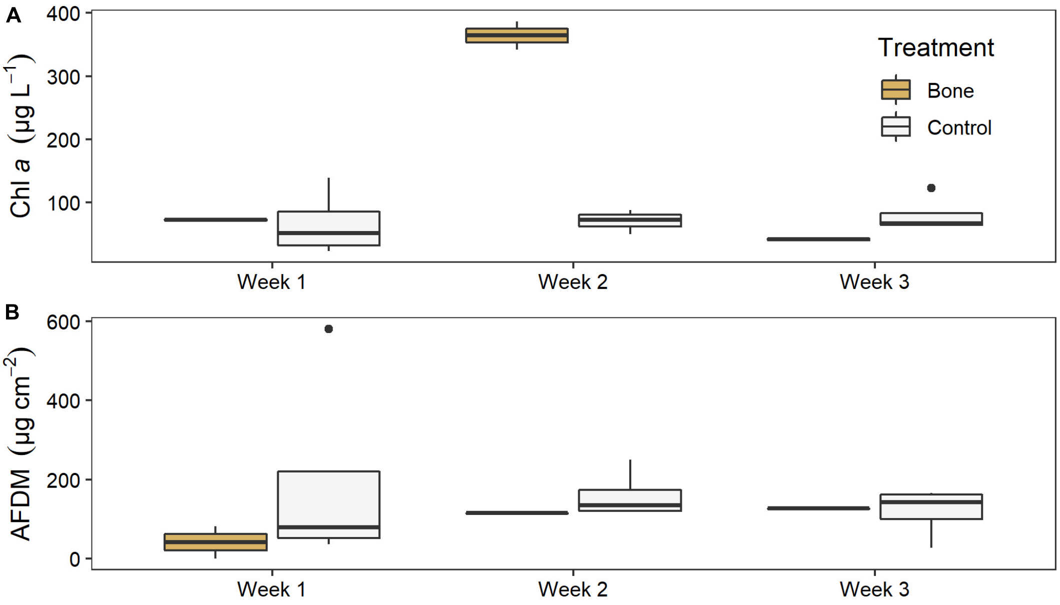

FIGURE 7 | (A) Water column chl *a* and **(B)** biofilm ash free dry mass (AFDM) on ceramic tiles in experimental streams with all gravel benthos (control treatment; *n* = 4) or half gravel-half bone benthos (bone treatment; *n* = 2) in Week 1, after equilibration and just before treatments were applied, and in Weeks 2 and 3 of the experiment, after treatments were applied.

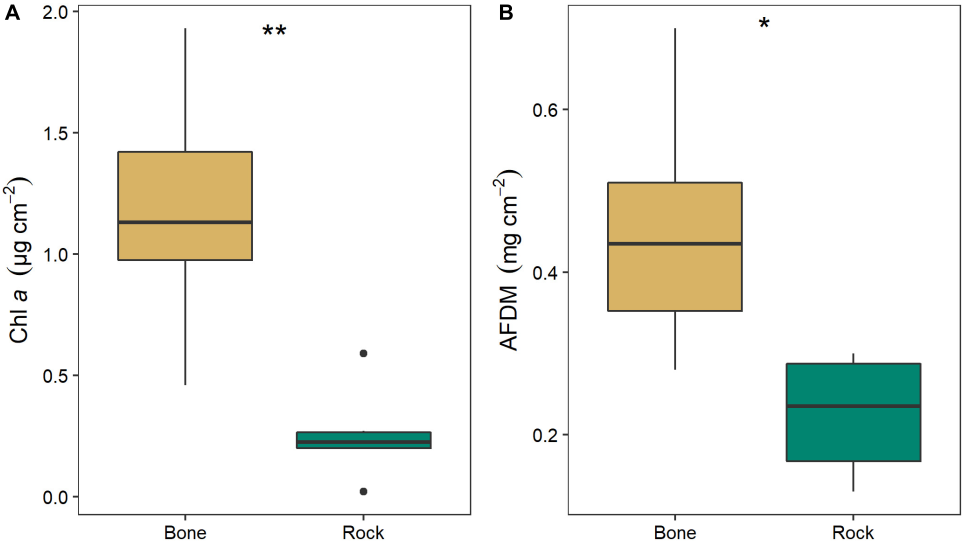

FIGURE 8 | Mean (± SE) values of **(A)** chlorophyll *a* and **(B)** organic matter as ash free dry mass (AFDM) on bone (*n* = 3) and rock (*n* = 3) substrates in the Mara River. Asterisks indicate significant difference, where $^{*}p < 0.05$ and $^{**}p < 0.01$.

indicate greater dissimilarities within groups than between groups (**Figure 9**). In 2017, unicellular algae were the most common, followed by colonial algae and diatoms. In 2018, diatoms were the most common, followed by filamentous and colonial algae.

Stable Isotopes

Biofilm on bones had a δ^{15}N similar to that of biofilm on rocks (0.4–1.7‰ difference) but a δ^{13}C that was much more enriched (4–10‰) (**Figure 10**). The δ^{13}C of biofilm on bones was much closer to the δ^{13}C of wildebeest bones themselves (1.6–3.7‰ difference). These data suggest biofilms on both rocks and bones may be obtaining N from the water column, but biofilms on bones may be obtaining some C from the bones themselves.

Sufficient differences between the three dominant basal resources at NMB in February 2014 (bone biofilm, rock biofilm, and CPOM) enabled us to parse their influence on aquatic consumers (**Table 2**). Mixing model results showed that bone

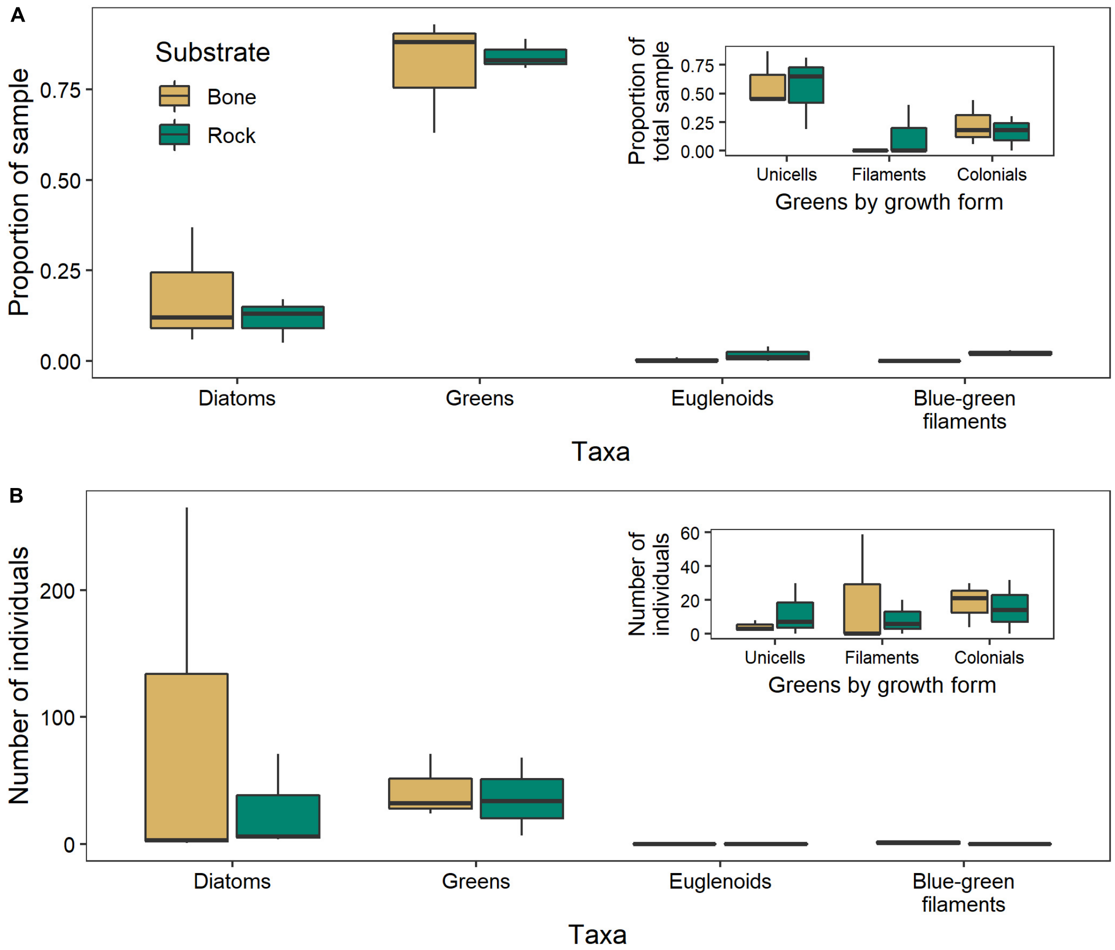

FIGURE 9 | Algal community composition on bone ($n = 3$) and rock ($n = 3$) substrates in the Mara River, where individuals are presented as **(A)** a proportion of 100 individuals counted in 2017, or **(B)** total number within 10 microscope fields in 2018. Green algae are parsed by growth form in the inset figures.

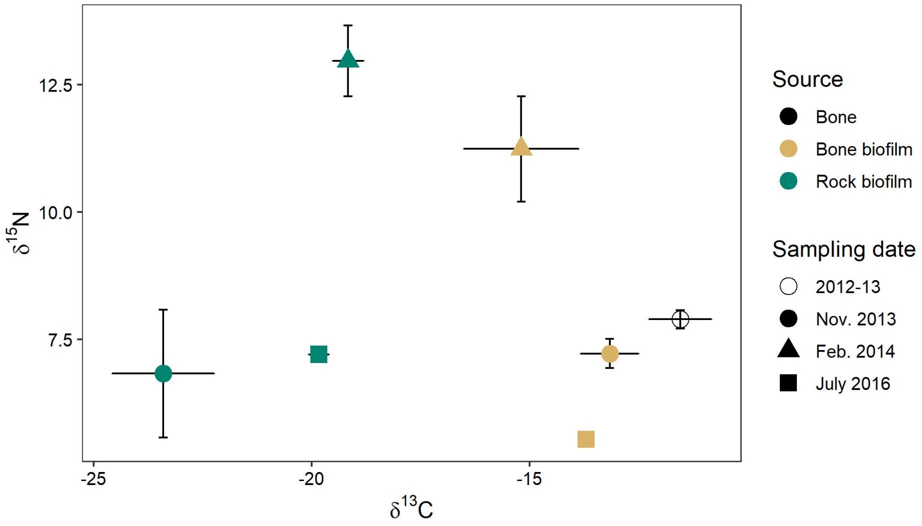

FIGURE 10 | C and N stable isotope signatures of wildebeest bone ($n = 8$) and biofilm on rock and on bone on three sampling dates ($n = 3$ of each at each time point) in the Mara River.

TABLE 2 | Stable isotope signatures of basal food web resources, aquatic macroinvertebrates, and fishes collected in February 2014, at the New Mara Bridge in the Mara River.

Category	*n*	δ^{13}C (mean ± SD)	δ^{15}N (mean ± SD)
Hippo feces	9	−14.7 ± 1.0	3.8 ± 1.0
Biofilms on rocks	3	−19.2 ± 0.6	13.0 ± 1.2
Biofilms on bones	3	−15.2 ± 2.3	11.2 ± 1.8
Aquatic macroinvertebrates	4*	−15.8 ± 1.8	7.8 ± 1.7
Fishes	16	−13.8 ± 1.1	10.2 ± 0.8

**Each macroinvertebrate sample represents one family comprised of a composite sample of ≤ 30 individuals.*

biofilm accounts for 19 ± 16% (mean ± SD) of assimilated tissue in macroinvertebrates and 24 ± 13% in fishes in the Mara River (**Figure 11**). These proportions are similar to that of rock biofilm for macroinvertebrates (21 ± 13%), but greater than rock biofilm for fishes (7 ± 6%). The remaining portion of both macroinvertebrate and fish diet is comprised of hippo feces, which accounts for nearly all of the particulate OM at this site. The results support an important contribution of bone; however, we note that the 95% Bayesian credible intervals are quite wide and overlap for most of the resources, indicating a reasonable amount of uncertainty in the contribution.

DISCUSSION

Wildebeest bones provide a distinct and complex resource in the Mara River, and given their abundance in this system, they may influence nutrient cycling, ecosystem production, and food web dynamics at the river ecosystem scale. Wildebeest bone is comprised of 22% C, 4% N, and 10% P, although stoichiometry and decomposition rates vary by bone type (**Table 1**). A mean of 15% of the initial mass of bones is relatively labile and decomposes over 80–120 days (**Figure 3**). Initial leaching releases a large amount of inorganic N relative to P (**Figure 5**). After this labile portion is gone, the more refractory material that is rich in P can persist in the system for decades, although mineralization and leaching of P continue to occur. For example, bones that had been submersed in the river for an unknown period of time still released SRP when placed in the experimental stream channels (**Figure 6**). The increase in SRP during week 2 of the experiment may have reflected either a pulse of SRP release in response to changing environmental conditions between the river and the experimental streams, or a steady release of SRP that declined in week 3 due to uptake. These data suggest that large mammal bones play a unique role as slow-release nutrient subsidies in this system.

Nutrient leaching from bones may stimulate increased production. In the experimental stream channels, the increase in SRP in the bone treatment in week 2 was coincident with five times more water column chl *a* but no change in tile AFDM (**Figure 7**), suggesting P from bones stimulated water column primary production. In the river, biofilms growing on bones had five times more chl *a* and two times more OM than biofilms growing on rocks (**Figure 8**), suggesting bones may support greater quantity and/or quality of biofilms. Increased biofilm growth on bones could be due to leaching of nutrients from the bones and/or greater surface roughness of bones as compared to rocks, which could provide increased surface area for growth (Bergey and Cooper, 2015). There did not appear to be a difference in algal composition on bones versus rocks (**Figure 9**).

The isotopic signature varied between biofilms on bones and those on rocks, with the δ^{13}C of bone biofilm being closer to that of wildebeest bones themselves (**Figure 10**). These data suggest some biofilm constituents may be able to utilize elements leaching from the bones, particularly C, which could have implications for bone biofilm quantity and quality. Mixing model analysis suggests aquatic macroinvertebrates and fishes assimilate a similar proportion of bone biofilm, which is equal to or greater than the proportion of rock biofilm assimilated (**Figure 11**). Given the likely much lower abundance of bones on the river bottom as compared to rocks, these data suggest consumers may be preferentially feeding on biofilms growing on bones. If so, that preference could be driven by higher density or quality of the biofilms growing on bones as compared to those growing on rocks.

Many of these analyses are based on small sample sizes, and we synthesize them here to stimulate areas for future research. Altogether, these data suggest bones may play an important and persistent ecological role in the Mara River, and they raise several over-arching questions about the potential ecological role of bones in aquatic ecosystems in general.

What Is the Magnitude and Frequency of Animal Bone Inputs to Aquatic Ecosystems?

The Mara River receives annual inputs of large mammal bones, and bones persist in this system over long timescales. The large input rate and slow decomposition rate yield a steady-state standing stock estimate of 4.4×10^6 to 5.6×10^6 kg of bones in the river, which is equivalent to the biomass of 49 blue whales. This estimate is likely high, as it assumes that the system is in equilibrium and input rates have been constant over time, and it is based on a conservative decomposition rate that does not account for animal consumption or mechanical breakdown. This estimate also does not reflect the potential distribution of bones downstream through the river system and into the Mara Wetland and Lake Victoria. Nevertheless, it reflects the magnitude of bone that can accrue in a system that receives large inputs of carcasses, particularly of large mammals. How typical is this of other aquatic ecosystems?

Animal bones can enter aquatic ecosystems through various pathways. Wenger et al. (2019) proposed a framework for classifying animal carcass inputs to aquatic ecosystems, in which inputs may be either autochthonous and continuous (e.g., annual mortality from aquatic animals), autochthonous and pulsed (e.g., mass mortality of aquatic animals), allochthonous and continuous (e.g., annual mortality from terrestrial animals that are transported in from the watershed), or allochthonous and pulsed (e.g., seasonal mortality from terrestrial animals that perish *in situ*). The wildebeest inputs to the Mara River represent

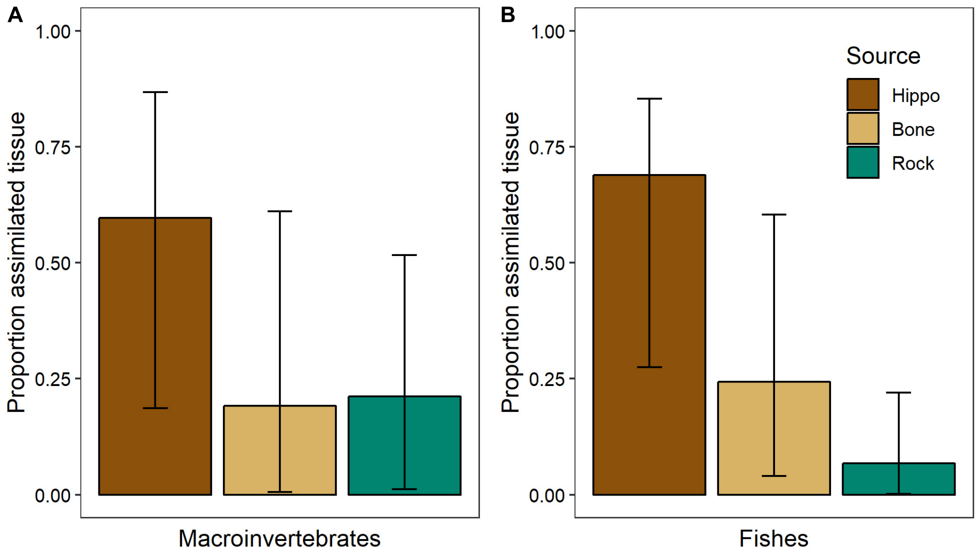

FIGURE 11 | Proportion of basal resources in assimilated tissue (mean and 95% Bayesian credible intervals) of **(A)** aquatic macroinvertebrates (*n* = 4 taxa, composite samples of 16–30 individuals/taxa) and **(B)** fishes (*n* = 3 taxa, 3–8 individuals/taxa) in February 2014 in the Mara River from hippo feces (*n* = 9), bone biofilm (*n* = 3), and rock biofilm (*n* = 3).

an example of allochthonous and pulsed, which has the potential to be one of the largest sources of inputs in certain systems. For example, research building on historical, anecdotal accounts suggests that large-scale inputs of bison bones may have been commonplace in the rivers of the American Great Plains as recently as the late 1700s. A mass drowning of bison in the Assiniboine River could have comprised ~50% of the annual P load for that river (Saindon, 2003; Wenger et al., 2019). It is unknown how the magnitude of these inputs would compare to those resulting from *in situ* mortality of aquatic vertebrates, such as fishes. However, allochthonous inputs from terrestrial animals that transport additional resources into the system are likely to have different and perhaps more pronounced ecosystem effects as compared to autochthonous ones (Subalusky and Post, 2018). Furthermore, the size of bones likely influences their stoichiometry and decomposition rate, such that larger bones may be expected to provide the slow release of nutrients we observed with wildebeest bones, whereas smaller bones may decompose more quickly (Nobre et al., 2019). Much work remains to be done to quantify the magnitude of animal carcass inputs from these four categories across ecosystems and over time and space. We expect that rates of allochthonous carcass inputs to aquatic ecosystems would be highest in higher order rivers, which both aggregate more from the watershed and may provide a cause of direct mortality for animals, and in landscapes with abundant populations of large mammals and particularly migratory herds.

How Bioavailable Are the Elements in Bones, and Over What Time Scales Do They Become Available?

Our data suggest most of the labile nutrients leach out of bone within a few months of deposition; however, they also indicate that the recalcitrant nutrients can continue to leach out of bone at longer time scales. Thus, bones have the ability to provide a slow-release nutrient subsidy to aquatic ecosystems, which lengthens the temporal scale at which we normally consider animal influences on nutrient cycling. What are the rates of P mineralization from the recalcitrant portion of bones, and what other elements may be leaching from the bones, such as calcium? How do these mineralization rates change across environmental conditions and over time? And how may the attachment of algae and microbes facilitate the erosion of bones through alteration of the boundary layer pH or scavenging of minerals? Much of the forensic and archeological study of bone decomposition has focused on bones buried in soil, and research suggests increasing soil temperature, moisture content and geochemistry are all important variables in driving microbial decomposition, although a great deal of variability occurs in bones even within the same site (Hedges, 2002; Christensen and Myers, 2011; High et al., 2015). In aquatic ecosystems, where less research has been done on bone decomposition, the decomposition process can be even more variable due to the large number of variables that can influence the process, including temperature, water depth, currents, dissolved oxygen, dissolved OM, substrate type, salinity, acidity, and insect and scavenging activity (Simon et al., 1994; Heaton et al., 2010). In lake and river ecosystems, bone burial in benthic sediments is likely to slow or stop decomposition. Studies of fish bones suggest 10–15% of bones may be permanently buried in lakebed sediments (Vallentyne, 1960; Schenau and De Lange, 2000; Vanni et al., 2013). How do decomposition processes vary across species and ecosystem types, and how bioavailable are elements throughout these processes?

As part of this study, we analyzed the C, N, and P composition of a bison skull that was recently recovered from Clear Lake, IA, United States, in order to understand how extended submersion in an aquatic ecosystem (e.g., over centuries to millennia) could influence bone composition in another species. Modern American bison (*Bison bison*) have been extirpated from that region for several 100 years; however, preliminary analysis based on the skull's shape and size suggests this may be a prehistoric specimen (Skinner and Kaisen, 1947). We conducted radiocarbon dating to determine the skull is 3,360 ± 25 years BP (^{14}C age); thus, this skull could have been in the water for 1000s of years. The bison skull had very similar proportion of OM (37%) and C:N:P stoichiometry (13.7 C: 4.3 N: 10.6 P by % mass) to the fresh rib and aged scapula bones from the wildebeest, suggesting that even bones 100s to 1000s of years old may still retain a fairly large proportion of organic material. In the case of the bison skull, burial in lake sediments in a cold region likely maintained its relatively intact condition.

Are Animal Bones Capable of Influencing Aquatic Ecosystem Function?

Altogether, our data suggest bones may provide important nutrient and microhabitat resources at local scales in this system. However, it is still unclear to what degree these effects scale up to influence the whole river ecosystem. To what degree can long-term mineralization of bone inputs support primary, and ultimately secondary, production in aquatic ecosystems? The answer likely depends upon both the magnitude of the inputs and the environmental context of the aquatic ecosystem (Subalusky and Post, 2018). In the Mara River, wildebeest bones contribute a substantial portion of the annual P flux from upstream (Subalusky et al., 2017). P flux is significantly higher at the site where wildebeest drownings occur, but only when carcasses are present, suggesting long-term leaching of bones does not significantly elevate P flux at the site scale (Subalusky et al., 2018). However, it may be readily assimilated and stimulate primary production, as we observed in the experimental streams. Indeed, water column chl *a* is higher at the site where drownings occur, although the degree to which that production is due to wildebeest inputs versus other drivers is still unclear (Subalusky et al., 2018). There also may be more localized hotspots of P availability by bone piles that could have ecological consequences. Whole ecosystem effects of wildebeest bones may be muted in the Mara River, where even larger resource subsidies from hippos have pronounced ecosystem effects (Subalusky et al., 2018). However, in systems with substantial inputs of large bones and low background nutrient loading, it is possible that bones could influence ecosystem function over long time scales. For example, in a study of ponds near early Arctic hunting communities, marine mammal bones from butchered carcasses were still evident in those systems 500- > 1500 years later, due to slow decomposition rates, and they still influenced pond water nutrients and production (Michelutti et al., 2013). Bones also may play an important role in providing structural habitat in rivers that otherwise lack it. Although this is not the case in the Mara River, which has a large degree of rock and boulder cover, it may be an important role of bones in other rivers flowing through grasslands.

What Comprises the "Osteophyton" Community, or the Biofilm Community That Grows on Bones?

In this study we analyzed the algal community at a coarse taxonomic resolution and found no differences between biofilms growing on bones and rocks, but do differences occur at a lower algal taxonomic resolution, or in the bacterial community? There are biofilm organisms, referred to as epibionts, that appear to specialize in colonizing surfaces of aquatic flora and fauna. For example, certain taxa of filamentous algae and diatoms have been found to specialize on turtle shells, likely due to the ability of those taxa to withstand frequent wetting and drying periods (Edgreen et al., 1953; Wu and Bergey, 2017). In some cases, the primary production of these epibionts may alter the net metabolism of their host (Lukens et al., 2017). Are there taxa that specialize on bones, either due to greater surface area roughness that enables better attachment or to their ability to utilize carbon or other elements leaching from the bones? Do these taxa provide a higher quality food resource for grazers? Are there aquatic species that directly consume bones, similar to rodents and hyenas in terrestrial ecosystems? Crocodilians can digest bones when consuming entire carcasses (Fisher, 1981). It is unclear whether bones would be directly consumed as a food resource, due to their relatively low caloric content, but they may provide other elements, such as calcium, that are otherwise limiting in the system.

CONCLUSION

Wildebeest bones appear to play an important ecological role in the Mara River system due to the quantity and frequency of input and their potential to influence short- and long-term nutrient cycling, production and aquatic food webs. Animal bones may play an important role in other aquatic ecosystems. In many ways, the role of bones may be similar to that of coarse woody debris in some systems, as both decompose over long time scales, provide food and structural habitat, and are capable of entraining finer particulates (Wohl, 2017). The role of bones may have been even more important before declines in animal populations, and declines in large mammals and migratory herds in particular, reduced the occurrence of large animals on the landscape. However, domestic animals may be replacing that role in some places. The biomass of domestic livestock and poultry now far exceeds that of wild mammals and birds (Bar-On et al., 2018). Most livestock carcasses are fully used or disposed of in controlled ways, and bones are often used as fertilizer or animal feed (Jayathilakan et al., 2012). However, catastrophic flooding, which has become increasingly common in some regions due to climate change, can lead to mass mortality of domestic livestock and transport of livestock carcasses into aquatic ecosystems. Animal bones from annual mortality may play a relatively small role in most aquatic ecosystems, but pulsed inputs from mass mortality events could be a substantial component of nutrient budgets (Wenger et al., 2019), and the bones could persist for decades or longer in the system. The role of bones in aquatic ecosystems is an area that deserves more study given the unique and long-lasting influence bones may have.

DATA AVAILABILITY STATEMENT

The datasets analyzed for this study and all code for the statistical analyses and figures are included in the **Supplementary Material**.

ETHICS STATEMENT

This study was carried out in accordance with the Yale University Institutional Animal Care and Use Committee Animal Use Protocol #2012-10734. This research was conducted under a research permit from the Government of Kenya and the National Council for Science and Technology (NCST/RRI/12/1/BS-011/25) in affiliation with the National Museums of Kenya.

AUTHOR CONTRIBUTIONS

All authors conceived of this study. AS, CD, ER, and DP collected the field data. LP collected the algal data. AS analyzed the data and wrote the initial draft of the manuscript. All authors assisted with writing and approving the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2020.00031/full#supplementary-material

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frontiers in Ecology and Evolution

EDITORIAL
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Editorial: Animal Mass Mortalities in Aquatic Ecosystems: How Common and Influential?

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Editorial on the Research Topic

Death and Decomposition in Aquatic Ecosystems

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INTRODUCTION

The prototypical animal mass mortality in aquatic ecosystems is the annual spawning migration of Pacific salmon (*Oncorhynchus* spp.) that can transport thousands of kilograms of labile organic resources to rivers and lakes. However, many other mass die-offs of vertebrates and invertebrates can strongly influence the structure and function of aquatic ecosystems. Here we discuss the spatial and temporal occurrence of mass mortality events in aquatic and riparian systems, *as informed by the preceding series of papers in this special issue*, and their influence on ecological processes. Fish in the families Salmonidae and Clupeidae undertake annual mass migrations that often result in their death in the ecosystem where they spawn, and therefore their macronutrients (C, N, P) and even micronutrients subsidize recipient ecosystems. In contrast, stressful conditions such as oxygen depletion or toxic algal blooms can result in unpredictable fish and mussel die-offs. Terrestrial animals ranging from insects to wildebeest also subsidize aquatic and riparian ecosystems during mass mortality episodes associated with migrations or adult emergence from or near water bodies. We propose a paradigm of "programmed vs. catastrophic" death whereby recipient ecosystems vary along a gradient in their history and capacity to process these subsidies based on the predictability and timing of the resource pulse. Such mortality events may be increasing in frequency and severity with global environmental change, and therefore a more robust understanding of their ecological effects is needed to inform theory and application.

BACKGROUND AND CONCEPTUAL FRAMEWORK

The collection of papers in this Special Issue of FEE, *Death and Decomposition in Aquatic Ecosystems*, documents a range of animal mortality events that have substantial documented ecosystem effects. The authors collectively demonstrate that animal death and decomposition, or contributions of dead organic matter from riparian zones, can have profound ecosystem effects independent of the predation process. Further, mass mortality events (MMEs) are significant phenomena that punctuate functional processes in terrestrial and aquatic ecosystems across the globe. In their seminal meta-analysis, Fey et al. (2015) assembled published reports of 727 MMEs based on a functional definition of an MME—namely, the rapid catastrophic die-off of multiple organisms in a single event. Fey et al. (2015) conclude that the largest magnitude MMEs result from starvation, disease, or multiple stressors, such as drought and long-distance migration in terrestrial ecosystems or eutrophication and oxygen depletion in aquatic environments. However, in this Special Issue, the authors also consider the "programmed" death of organisms that results from life-history traits, such as spawning migrations or life stage transitions (cf. Lamberti et al., 2010).

In general, reports of MMEs have been increasing in frequency since reasonably reliable records began to be kept in the 1940s. Fey et al. (2015; see their Figure 1) cataloged die-offs of five vertebrate animal groups—mammals, birds, amphibians, reptiles, and fish—along with the general category of invertebrates. Fish die-offs represented more than half of all reported MMEs, whereas reptile and invertebrate die-offs were least common or least reported. The latter aspect of reporting is significant, since mortality in taxa such as fish or mammals may be more likely to be documented than die-offs of insects or other invertebrates. Regardless, die-offs in most groups showed an increasing frequency of occurrence over the past 75 years, involving an increasing number of individuals for most taxa (Fey et al., 2015; see their Figure 2). The apparent increase in MMEs over time may be due to growing interest in the phenomenon by the scientific community but also rapidly increasing publication activity over that period, as the frequency of MMEs broadly tracks the number of literature citations for particular animal groups. Furthermore, as global change accelerates, interest in MMEs has increased related to habitat loss, climate change, biological invasions, and animal overharvest (Sala et al., 2000).

As largely unpredictable events, MMEs often lack quantitative information on their magnitude beyond rough estimates of the number of individuals that died. For example, few studies report metrics important to assess mass contributions to ecosystems, such as total biomass (wet or dry) or elemental composition of the carrion. Therefore, we collected data from several MMEs for which relatively robust information was available on the mass of animal carrion resulting from the event and the stoichiometry of the major nutrients (C, N, P) in the dead tissue, either from the original or related publications. For seven such MMEs (**Figure 1A**), the animal dry mass of these events ranged from 1.7 mT (dry mass) for an episodic die-off of *Corbicula* clams in a 10-km reach of the Broad River, GA, USA, to 301 mT for wildebeest in the Mara River of the Maasai Mara in Kenya, Africa. Other taxa found in specific systems show a broad range in mass contributions to aquatic or riparian ecosystems (**Figure 1A**). The stoichiometry (i.e., C:N:P ratio) of this biomass also varied substantially among taxa (**Figure 1B**). After normalizing to P (i.e., P set at 1.0), wildebeest had the lowest C:N:P ratio of 21:4:1, suggesting a relatively rich P source, whereas cicadas had the highest ratio at 195:33:1, suggesting a depleted P source. The two insect taxa (cicadas and midges) had the highest ratios overall likely because of the high C content and mass of the exoskeleton relative to internal tissues. Fish had overall favorable stoichiometry relative to the Redfield ratio (i.e., 106:16:1), varying from 48:8:1 for cichlids to 131:17:1 for pink salmon.

Fish are the most common MMEs recorded in the literature (Fey et al., 2015), in part due to the predictable annual spawning migrations of some taxa that exert high physiological stress or genetically-determined death. For example, annual salmon spawning migrations in rivers throughout the world generate a large and predictable pulse of dead biomass in those ecosystems. Marine fish in the family Clupeidae (e.g., alewife, shad, herring) also undergo annual spawning migrations, often resulting in substantial mortality in freshwater ecosystems (Durbin et al., 1979). In contrast, fish die-offs from environmental stressors (e.g., eutrophication, elevated temperature, dissolved oxygen depletion), sometimes exacerbated by metabolism of the fish themselves, can deposit unpredictable pulses of biomass in space and time such as with cichlid fish in a Brazilian reservoir (Starling et al., 2002) or have strong ecosystem effects as for salmon in southeast Alaska streams (Sergeant et al., 2017). Even without mass mortality, Nobre et al. argue in this issue that fish (gizzard shad) carcasses in Ohio (USA) lakes are net nutrient sources to the water column over decadal scales even while live fish are net nutrient sinks at an annual scale. They compute that an amazing 99% of the nitrogen and phosphorus from these fish is efficiently mineralized in the water column and thus available for uptake by plankton.

Aquatic invertebrates are often overlooked sources of ecosystem alteration due to synchronous mass mortalities. Sedentary organisms can be most sensitive to human alteration of ecosystems. For example, MMEs of freshwater unionid mussels, many of which are imperiled, can have significant and lasting ecological effects including losses in biofiltration, nutrient cycling, and nutrient storage (e.g., Vaughn et al., 2015). In this issue, DuBose et al. warn that unionid dieoffs in rivers are accelerating due the dual impacts of drought and temperature increase resulting from climate change. While such dieoffs can produce short-term beneficial nutrient pulses, declines in these long-lived organisms can result in permanent loss of ecosystem function.

Further, die-offs of invasive bivalves (e.g., *Corbicula* spp., dreissenid mussels) can affect both instream and riparian nutrient dynamics and scavenger communities (Mouthon, 2001; Novais et al., 2015). Bivalve death also elicits unique long-term effects through the mass availability of recalcitrant shells that can take decades to decompose and offer new habitat availability and slowly released sources of calcium and phosphorus (e.g., Wenger et al., 2018). In this issue, McDowell and Sousa suggest that invasive mussels are less able to tolerate extreme abiotic conditions (e.g., in hydrology or temperature) than are native mussels and therefore are more prone to mass dieoffs. Such dieoffs release nitrogen pulses and shells alter habitat, while decomposition exerts oxygen stress on other organisms resulting in cascading ecosystem impacts.

Insects often undergo synchronous mass mating emergences followed quickly by egg-laying and death. For example, emergent insects from soils (cicadas in North American gallery forests; Whiles et al., 2001; Yang, 2004) and lakes [chironomid midges in Lake Mývatn, Iceland (Dreyer et al., 2015); chaoborid midges in Lake Malawi, Africa (Irvine, 2000)] produce large quantities of biomass that is disseminated in surrounding ecosystems and sometimes even used as a human food subsidy (Williams and Williams, 2017). In reality, the pathway of organic matter exchanges between aquatic and terrestrial ecosystems is bidirectional. In this issue, Entrekin et al. describe how declines in carbon inputs to streams (in

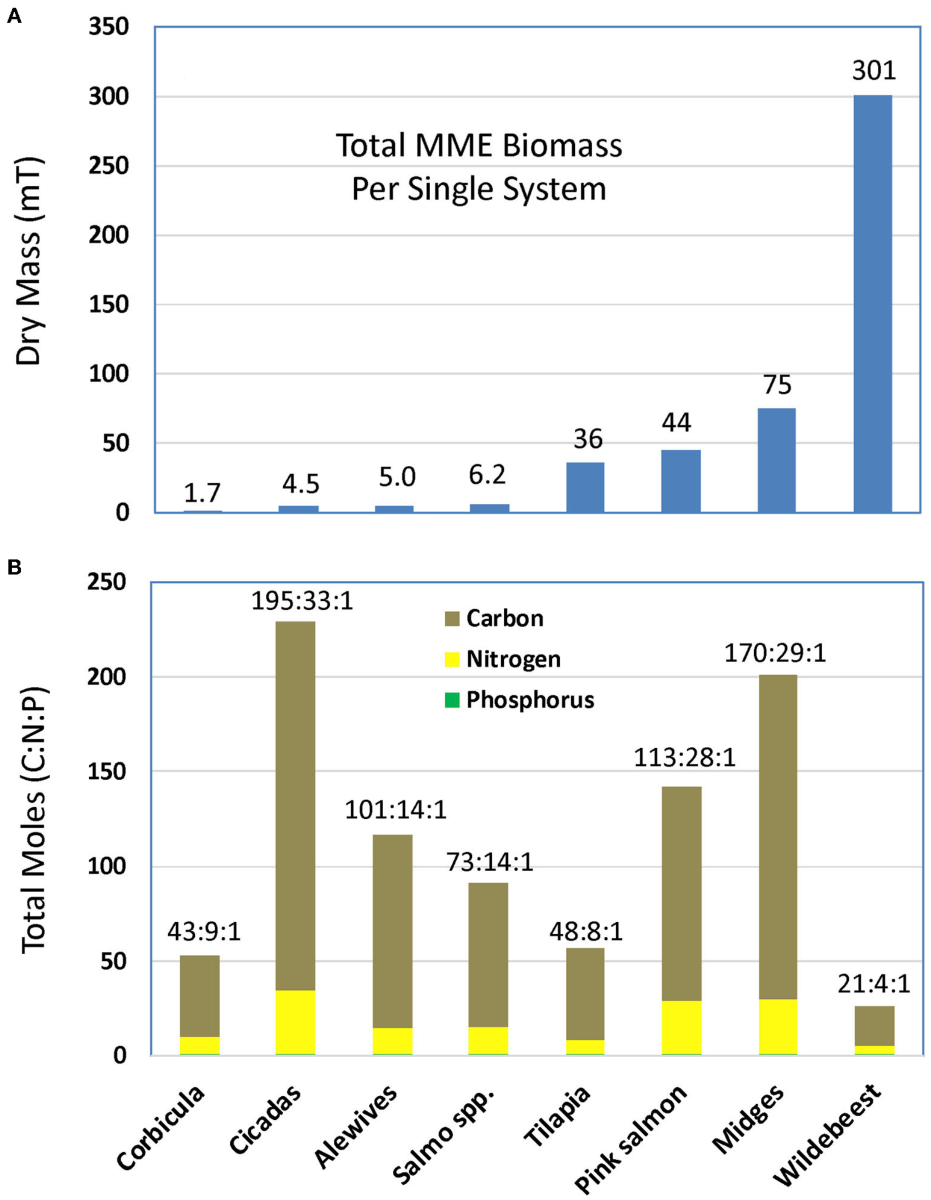

FIGURE 1 | (A) Total dry mass (mT) and **(B)** molar stoichiometry of carbon (C), nitrogen (N), and phosphorus (P) for a single mass mortality event (MME) in a single system (e.g., river, lake) of Asiatic clams *Corbicula fluminea* (McDowell et al., 2017), periodical cicadas *Magicicada cassini* (Whiles et al., 2001), alewives *Alosa pseudoharengus* (Durbin et al., 1979), Atlantic salmon and brown trout *Salmo* spp. (Lyle and Elliott, 1998), cichlid fish *Tilapia* spp. and *Oreochromis* spp. (Starling et al., 2002), pink salmon *Oncoryhnchus gorbuscha* (Helfield and Naiman, 2001), chironomid midges *Chironomus islandicus* and *Tanytarsus gracilentus* (Dreyer et al., 2015), and wildebeest *Connochaetes taurinus* (Subalusky et al., 2017). Total biomass or stoichiometric ratio is given above each bar.

the form of large wood and organic debris) can cascade into reductions in ecosystem function. When large wood was added back to a temperate stream, carbon flow through the food web was stimulated, resulting in a 20% increase in macroinvertebrate production. Therefore, donations of organic matter between aquatic and terrestrial ecosystems represent a dynamic process that often interfaces through the activities of invertebrates.

SELECTED EXAMPLES OF MASS MORTALITY EVENTS

As demonstrated in this Special Issue, the examples of MMEs are diverse and global, but all have the potential to pulse animal carrion into ecosystems and potentially to deliver that biomass across traditional ecosystem boundaries. Below we provide three examples of MMEs that either can drive ecosystem change or respond to environmental change relevant to freshwater ecosystems. These examples highlight several of the topics explored in this Special Issue augmented with other published accounts of MMEs.

Spawning Runs of Pacific Salmon

In aquatic ecosystems, Pacific salmon (*Oncorhynchus* spp.) provide a classic example of mass mortality and have been the topic of hundreds of studies over the past two decades (see reviews by Schindler et al., 2003; Janetski et al., 2009), reflecting their ecological, economic, and sociocultural importance in the northern Pacific rim (Gende et al., 2002). Most salmon species typically undertake a predictable (i.e., spatial, temporal) annual migration from the ocean to freshwater for a single semelparous spawning event that culminates in death. When adult salmon return to freshwater they deposit so-called "marine-derived nutrients" in natal ecosystems as gametes, carcasses, and excretory products, often with pervasive and dramatic ecological effects (Tiegs et al., 2011).

The most dramatic aspect of the salmon mass spawning event is simply the magnitude of the flux (Larkin and Slaney, 1997) and the dependency of ecosystems and associated organisms upon this material (Cederholm et al., 1999). In Alaska (USA) alone, since 1975, an average of approximately 150 million Pacific salmon have been commercially harvested annually (most offshore), producing over 300 million kg of fish for human and animal consumption [Alaska Department of Fish Game (ADFG), 2019]. Individuals that are harvested do not contribute to the mass die-off following spawning and therefore to the resource subsidy to freshwater, estuarine, and adjacent terrestrial ecosystems. However, the very magnitude of this number represents a substantial movement of carbon and nutrients from the ocean to the terrestrial environment. Specific data for the total abundance of dead spawning salmon do not exist, but rough estimates place the number at 150 million salmon spawners in Alaska, about equal to those harvested commercially (Munro, 2018).

Given the putative stoichiometric ratio of an average fish (**Figure 1B**; see also Larkin and Slaney, 1997), this migration annually transports and deposits an estimated 150 million kg C, 15 million kg N, and 2 million kg P as highly labile compounds into freshwaters. Recently, we have learned that micronutrients from these carcasses also may be important to recipient ecosystems (Currier et al., 2020). Moreover, this predictable and sustained flux enters systems that are otherwise nutrient-poor and at a time of year (often autumn) when productivity is limited by declining temperatures and light levels (Gende et al., 2002). In this issue, Harding et al. discuss how the effects of these carcass resource subsidies on spawning rivers, surrounding riparian forests, and their estuaries can vary with watershed landscape structure, salmon species that are spawning, size of the run, and the vertebrate scavengers in the local area. In their British Columbia (Canada) watersheds, bears and wolves were the major vectors of salmon into riparian zones, while salmon density and watershed size drove the "leakage" of carcasses into estuaries.

The vision of salmon runs feasted upon by bears and other macro-predators elicits awe in observers and notions of short food chains. In this issue, however, Larson et al. use molecular approaches to explore the microbiome in salmon streams and make the case that salmon decomposition subsidizes the microbial loop. Salmon do this by providing essential nutrients but also by introducing novel microorganisms into the ecosystem. This process is facilitated by macroinvertebrates that graze on carcasses and then harbor in their guts, and potentially transfer, novel microbes during their feeding and excretion. In this issue, Rüegg et al. further point out that spawning salmon are both a source of nutrients (from their bodies) but also a source of disturbance (from nest-building) to benthic biota. Biofilm often responds with increases in biomass early in the salmon run, declines during active nest building, and then another increase during carcass decomposition, as verified by stable isotopes.

Thus, salmon mass die-offs connect multiple ecosystems, and represent a potentially important "resource injection" for both aquatic and terrestrial organisms, plant as well as animal, at a critical time in these ecosystems. However, evidence is increasing of a potential "dark side" of salmon migrations especially where they have been introduced as a sport fish. In this issue, Gerig et al. review a body of recent literature documenting how Pacific salmon introduced into the Laurentian Great Lakes now accumulate, transport, and deposit contaminants (especially persistent organic pollutants, or POPs) in Great Lakes spawning tributaries where they have become naturalized. Riverine fish that do not migrate now carry the toxic imprint of these salmon via food web transfer, particularly through egg consumption. This POP biotransport is now also being reported in remote southern hemisphere rivers (e.g., Patagonia) where Pacific salmon have been introduced (Montory et al., 2020).

Migration and Seasonal Death in Wildebeest

A dramatic example of an MME involving a terrestrial herbivore providing a whole-body resource subsidy to an aquatic environment is the mass migration of wildebeest (*Connochaetes taurinus*) in Kenya and Tanzania of eastern Africa. A wide range of biomass inputs from MMEs exist (**Figure 1A**), but wildebeest fatalities during their multiple crossings of the Mara River yield the highest biomass contribution to a single system of the studies yet conducted (Subalusky et al., 2017). In one of the largest annual migrations on Earth, approximately one million wildebeest migrate through the Serengeti and arrive in the Maasai Mara region of Kenya in summer where they remain until late autumn. Large herds regularly cross the Mara River, and the mortality associated with crossings is influenced by a combination of recent precipitation (driving river discharge), river geomorphology, herd size, and riverine predators.

With a mean wildebeest body wet mass of 175 kg, mass drownings deposit an estimated 570–1622 metric tons of biomass (156–446 metric tons dry biomass) in the river annually (Subalusky et al., 2017) with an elemental composition of 36%

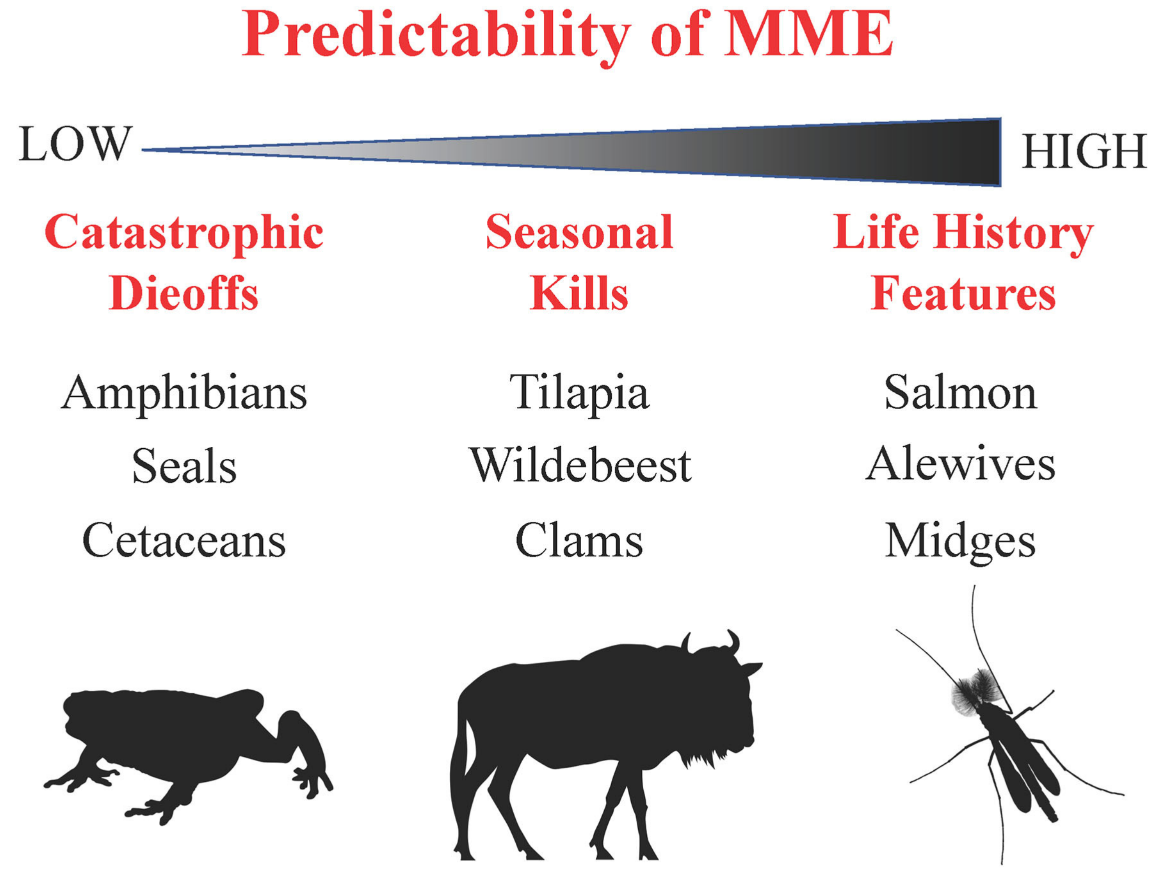

FIGURE 2 | Conceptual diagram depicting a predictability gradient of mass mortality events, and therefore possible ecosystem effects, ranging from catastrophic die-offs (left-hand side) to programmed death (right-hand side) with example organisms.

C, 8% N, and 4% P by mass. Carcasses tend to accumulate in regions of slow flow, where they fuel nutrient cycling and food webs as they decompose. During this MME, more dead biomass is deposited per unit river length than even during a typical Pacific salmon run. Scavengers and decomposers that exploit this resource are very diverse and include fish, crocodiles, hippopotamus, vultures, and other birds that congregate on the carcasses. In this issue, Subalusky et al. highlight the large pool of phosphorus represented by the massive amount of wildebeest bones deposited in the river that can persist for decades. In a two-stage process, bones leach mainly nitrogen initially, but then leak phosphorus for decades. The rich biofilm that grows on these bones supports 19% of macroinvertebrate and 24% of fish growth in the river, as revealed by stable isotopes. In this sense, Subalusky et al. fittingly refer to the Mara River as a "river of bones" that brings added attention to importance of bones in other ecosystems.

The influence of vertebrate death and decomposition on aquatic ecosystems depends on the magnitude, history, and recurrence of the mortality event, as emphasized by Benbow et al. in their introduction to this issue. Salmon and wildebeest represent reasonably predictable subsidies of carcass material to aquatic ecosystems, but even these are dependent on numbers of individuals and river hydrology during the migratory season. Benbow et al. note that the high nutrient and caloric density of carrion makes this material particularly important to energy-limited food webs (e.g., rivers) that may otherwise be dependent on lower-quality plant detritus to fuel production. Such necrophagy can have profound influences on many aquatic ecosystems.

Mass Emergence and Death in Insects

Insect emergence can be a substantial source of biomass for associated ecosystems, especially during synchronous emergences. These contributions are bidirectional between aquatic and terrestrial ecosystems, with riparian areas providing transitional habitats. Cicadas (Hemiptera) undergo cyclical mass emergences (i.e., nymph to winged adult) from forest soils (Yang, 2004), and can contribute to aquatic and riparian processes during death and decomposition. For example, the mass emergence (150 individuals m^{-2}) and death of 17-year periodical cicadas (*Magicicada cassini*) represented a substantial N flux (up to 3g N m^{-2}) to riparian gallery forests of the Kings Creek watershed in Kansas, USA (Whiles et al., 2001). These cicadas had high relative C content (C:N:P = 195:33:1) likely due to chitin in their exoskeleton, reasonable N content, and low P content. Therefore, direct consumption or decomposition would represent a good N source but relatively poor P source. In freshwater ecosystems, many species of aquatic insects emerge *en masse* (e.g., Ephemeroptera), and then mate and die in a matter of hours to days. These aquatic mass emergences and subsequent deaths are important resource subsidies to adjacent riparian forests as well as aquatic food webs themselves during egg-laying (Baxter et al., 2005). The stunning mass emergences of *Hexagenia* mayflies from the Mississippi River and Great Lakes (USA) are visible on weather radar and can inject up to 88 billion individuals and 3,000 mT of biomass into the airspace and later back to ecosystems (Stepanian et al., 2020). However, recent declines in mayfly abundance have caused grave concern about loss of these resource subsidies and other ecosystem services that they provide.

Mass emergences of chironomid midges (Diptera) from lakes are common phenomena that can serve as a link between aquatic and terrestrial ecosystems. Dreyer et al. (2015) estimated midge (*Chironomus islandicus* and *Tanytarsus gracilentus*) emergences and fluxes along the shore of Lake Mývatn ("midge lake") in Iceland. As larvae, these midges feed on detritus and biofilm on the lake bottom. After pupating, individuals float to the surface, emerge as adult flies, and mate in swarms near aquatic habitat. After egg-laying, dead midges contribute biomass to both the lake and shore, mostly within 100 m of the lake, thereby providing an aquatic to terrestrial resource subsidy. In Lake Mývatn, mass emergences occur in late spring to early summer with periodic (5–8 years) cycles of high abundance. Total annual N flux to shores was estimated to be 12 kg per hectare, increasing the available nitrogen in the soil surrounding the lake, and total annual P input was ∼1 kg per hectare. Return inputs to Lake Mývatn are more difficult to estimate, but likely also represent a return flux of nutrients to the lake. Hoekman et al. (2019) show that terrestrial arthropod predators decline in abundance in plots where midges are excluded, and display stable isotope enrichment when allowed access to midge prey. The availability of emergent midges may also coincide with bird migrations to the lake. In addition to chironomid midges, lakes of Africa also have substantial mass emergence events of phantom midges (Diptera: Chaoboridae) and some mayflies (Ephemeroptera: Polymitarcyidae) that provide significant energy and nutrients to adjacent landscapes (Okedi, 1992; Allison et al., 1996).

GENERAL PARADIGM

In summary, the examples provided suggest that mass mortalities exist along a predictability gradient, where programmed death is predictable and repeatable, whereas catastrophic die-offs are unpredictable and sporadic (**Figure 2**). Intermediate are seasonal die-offs that have varying magnitude from year to year, if they occur at all, and can respond to variable environmental conditions. However, except for a few notable exceptions (e.g., Pacific salmon, wildebeest), we know surprisingly little about the ecosystem impacts of these mortality events, many of which are linked to migrations that are declining at a global scale (Wilcove and Wikelski, 2008). Poorly detailed observations of MMEs, largely a result of their unpredictable nature, rarely allow us to compute mass-balance estimates. Even when care is taken to note the number or biomass of the dead, limited analyses of animal C:N:P exist from which to draw strong ecological conclusions about stoichiometric influences. Detailed biomass estimates and decomposition studies, followed through time, may allow us to understand the fate of carcass material left in the environment, and the pathways that are followed within and beyond an ecosystem. Furthermore, food web analysis, specifically determining the stoichiometric ratios, biomass, and pathways by which material flows, would be valuable in determining the fate of ecological subsidies provided by the event. Clearly, when thoroughly investigated, mass mortality events could drive ecosystem-wide changes in nutrient cycling, productivity, distributional ranges, and biodiversity. Ongoing global environmental change may drive systems toward less predictable mass mortality or changes in the magnitude and frequency of these important events.

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GL, NL, DC, and MEB contributed to the writing and revision of this manuscript. MAB analyzed data from the published literature and produced **Figure 1**. All authors contributed to the article and approved the submitted version.

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