Received: 17 September 2021 | Revised: 26 January 2023 | Accepted: 6 February 2023

DOI: 10.1111/fwb.14066

Freshwater Biology WILEY

ORIGINAL ARTICLE

Experimental additions of allochthonous dissolved organic matter reveal multiple trophic pathways to stimulate planktonic food webs

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Abstract

1. There is still considerable debate as to whether the allochthonous (terrestrial) dissolved organic matter (DOM) mobilised during large flow events plays an important role in supporting secondary production in riverine food webs. Understanding how food webs respond to large pulses of terrestrial DOM (tDOM) is important for conceptualising the relative importance of energy sources supporting food webs.
2. A mesocosm experiment (1,000 L) using three concentrations of leachate (1, 4, 8 mg C/L and a control) made from floodplain DOM was run for 34 days in a dam filled with water from Gunbower Creek in Northern Victoria, Australia. Nutrients, phytoplankton, zooplankton and stable isotope ($\delta^{13}C$ signatures) data were collected to examine how floodplain nutrients affected growth and community structure within the lower food web typical of an Australian lowland river.
3. All leachate additions led to very high concentrations of zooplankton and mixotrophic algae compared to the control. Mixotrophs dominated the algal biovolume of all leachate additions until Day (D)20 and appeared to drive changes in $\delta^{13}C$ signatures of particulate organic matter (POM) which were significantly related to changes in zooplankton $\delta^{13}C$ signatures. tDOM additions did not significantly suppress obligate autotroph growth which also appeared important as a food source, as reflected in the $\delta^{13}C$ signatures of zooplankton and POM after D10.
4. These results show the ability of phytoplankton and zooplankton communities in lowland rivers to respond quickly to changes in resource availability and quality. Mixotrophs appeared to provide an important trophic link between allochthonous carbon and primary consumers, and increased complimentarily to autotrophic production. This resulted in large net increases to phytoplankton biovolume and potentially played a significant role in driving changes in zooplankton growth.
5. We suggest that allochthonous DOM may be highly bioavailable and support production through several different trophic pathways, offering a large boost to production via both autotrophy and heterotrophy. Furthermore, we contend that

mixotrophy may be an important pathway for allochthonous organic matter to enter riverine food webs and support secondary production.

KEYWORDS
allochthonous energy, food webs, mesocosm, mixotrophy, zooplankton

1 | INTRODUCTION

Energy in lowland riverine food webs can be conceptualised as originating from two primary sources: within system photosynthesis, primarily from phytoplankton (autochthonous production), and external terrestrial organic matter (allochthonous production). The majority of allochthonous organic matter is delivered to lowland rivers during flood events as increased hydrological connectivity with floodplain environments mobilises large amounts of terrestrial dissolved organic matter (tDOM; Westhorpe & Mitrovic, 2012). These allochthonous energy pulses have been hypothesised as important resource subsidies which sustain riverine food webs and the productivity of aquatic ecosystems (Burford et al., 2008; Junk et al., 1989). However, there is considerable debate as to whether allochthonous DOM supports secondary production, especially at higher trophic levels in freshwater food webs (Brett et al., 2012; Cole et al., 2011; Thorp & Delong, 2002). Much of the debate to date has focused on resource quality, whereby allochthonous organic matter is considered to be of a lower quality than autochthonous organic matter, as a result of its higher recalcitrance and lower fatty acid content (Brett et al., 2009). Furthermore, for tDOM to support higher trophic levels several extra links in the food chain are required to reach higher trophic levels (owing to uptake via the microbial loop), reducing its carbon transfer efficiency compared to the autochthonous energy pathway (Brett et al., 2009; Sommer et al., 2002).

Despite the prevailing view that tDOM is a poor-quality carbon source, the quality of allochthonous DOM is variable (Berggren, Laudon, et al., 2010; Berggren, Ström, et al., 2010) with environmental conditions such as rainfall events and periods since terrestrial wetting greatly affecting the bioavailability of allochthonous organic matter (Baldwin et al., 2016). Local conditions such as riparian vegetation cover, stream width, location within catchment and state of the flow regime also affect tDOM quality and subsidy potential (Bunn et al., 2003). This heterogeneous composition of tDOM leads to a portion that is immediately bioavailable (Baldwin, 1999; Hitchcock & Mitrovic, 2013), and other more recalcitrant portions that may be more slowly degraded and assimilated over a period of months (Gawne et al., 2007). The two portions may both play important roles in supporting food webs because an initial pulse of microbial production during flood events followed by a slow release has been hypothesised to maintain food-web stability during variations in primary productivity (Wetzel, 1995). Furthermore, the high volume of tDOM mobilised during flood events may mean that despite low transfer efficiencies, there may still be significant subsidies to higher trophic levels (Pace et al., 2004; Tanentzap et al., 2017). Ultimately, the impact of allochthonous subsidies on riverine food webs is likely to be highly dependent on the quality and quantity of tDOM, coupled with the local environmental conditions during flow events, making the potential for tDOM subsidies to be highly variable between ecosystems (Marcarelli et al., 2011).

Thorp and Delong (1994) suggested in their Riverine Productivity model, that energy mobilised by phytoplankton through photosynthesis and direct input of riparian carbon forms the base of lotic food webs. However, during flood events in floodplain rivers, when tDOM concentrations are high, bacterial production may become dominant as it is decoupled from autochthonous production whereas phytoplankton growth is simultaneously suppressed by low light availability (Carney et al., 2016; Drakare et al., 2002; Jansson et al., 2007). This potential dominance by bacterioplankton (Jansson et al., 2000) also can lead to a consequent reduction in the stoichiometric quality of autotrophs as a result of nutrient limitation, leading to reductions in food quality for metazoan consumers (Danger et al., 2007).

Emerging evidence suggests that mixotrophic microalgae may be able to obtain energy through both photosynthesis and phagotrophic consumption of bacteria, and efficiently link allochthonous DOM to higher consumers (Flynn et al., 2013; Hansson et al., 2019). Mixotrophy may be advantageous during or following flood events when bacterial abundance may be high and light levels are reduced owing to coloured DOM and suspended sediments (Kamjunke et al., 2007). Further, mixotrophs are considered an ideal food source for zooplankton as a result of their nutrient stoichiometry being closer to that required by zooplankton (Hansson et al., 2019; Katechakis et al., 2005). Thus, mixotrophic microalgae may provide an alternate pathway through which allochthonous carbon can support higher trophic levels (Flynn et al., 2013).

Zooplankton are the main consumers of planktonic organisms in freshwater food webs, making them a crucial link between the lower food web and higher consumers (Kobayashi et al., 1998). The level of allochthonous support of zooplankton may vary significantly with different feeding behaviours (Berggren et al., 2014). Experimental evidence has found that *Daphnia* sp. were unable to survive on bacteria-fed tDOM alone and required phytoplankton for basic survival and reproduction (Brett et al., 2009). However, subsequent studies have shown that bacteria can supplement up to 50% of the phytoplankton diet with no adverse effects (McMeans et al., 2015; Wenzel et al., 2012). Furthermore, Degerman et al. (2018) found that additions of glucose lowered food-web efficiency but still resulted in a net increase in zooplankton production. Mesocosm studies using tDOM additions, in the form of leachates, have reported increased rotifer and copepod

production in the Namoi and Bega Rivers, Australia (Hitchcock et al., 2016; Mitrovic et al., 2014). Hitchcock et al. (2016) further found calanoid $\delta^{13}C$ signatures were more similar to those of the leachate treatments as leachate concentration increased, indicating that these zooplankton were assimilating the added allochthonous carbon. Field data using $\delta^{13}C$ stable isotopes also has indicated potentially high allochthonous carbon use in higher trophic levels, with some zooplankton using up to 50% (Pace et al., 2004) of carbon from allochthonous sources and fish up to 20% (Jones et al., 2018). These studies highlight the variable and potentially significant role of allochthonous carbon processing by bacteria in supporting metazoan freshwater food webs.

This study aimed to better understand how inputs of allochthonous DOM influence the structure and production of planktonic aquatic food webs. We used mesocosms, similar to those of previous studies (Faithfull et al., 2012; Hitchcock et al., 2016; Karlsson et al., 2007; Mitrovic et al., 2014), to experimentally manipulate a riverine food web with tDOM leachate additions. To understand the role of tDOM on riverine food webs we tested three hypotheses:

1. Pulses of allochthonous DOM would increase zooplankton production relative to the concentration of the DOM added, with higher concentrations supporting more production.
2. That the $\delta^{13}C$ values of zooplankton would be closer to the leachate $\delta^{13}C$ values than the control, reflecting tDOM assimilation by the end of the experiment.
3. Autochthonous production would be reduced by pulses of tDOM, such that mixotrophs would dominate the algal community.

2 | METHODS

2.1 | Study site

Gunbower Creek (35°47′45.4″S, 144°13′16.0″ E) is a major tributary of the Murray River in South-Eastern Australia. Floodplain inundation, resulting in large pulses of allochthonous organic matter, is relatively common on Gunbower Creek during and after large flow events (Nielsen et al., 2016). River redgums (*Eucalyptus camaldulensis*) are the most common species in the riparian vegetation in the area which is typical of floodplains on Australian lowland rivers (Westhorpe & Mitrovic, 2012).

2.2 | Mesocosms and leachate addition

The mesocosm experiment was performed in a specialised PVC-lined dam (2.5 m deep, 10 m wide, 50 m long) located next to the Gunbower Creek. The dam was filled with the creek water using irrigation pumps 5 days before the experiment and topped up again the day before filling the mesocosms. Pumps installed at either end of the dam circulated water and prevented stratification throughout the waterbody during the experiment.

The mesocosms were built using bulk bags (90 cm ×90 cm wide, 160 cm deep) with a waterproof PVC liner. Mesocosms were secured into three groups of four, representing the three treatments and control in triplicate. Each group of mesocosm bags was held together using a reinforced PVC pipe framework 200 cm × 200 cm × 200 cm with the mesocosm bags held firmly inside this framework using rope, similar to system of Hitchcock et al. (2016). Each frame was submerged until only the top 30 cm of each mesocosm bag was above water and held in the water column using floats, resulting in a total volume of 1,000 L for each mesocosm. The frame of each group was anchored at four points to both sides of the dam to stop any potential drifting from wind. The grouped mesocosms also were tied to each other to make a continuous line to minimise any variation in light environment. To stop birds and organic detritus entering the mesocosms they were covered with wire (1 cm^2 aperture).

In order to prepare the leachate, floodplain materials were collected from eight randomly distributed 1 m^2 quadrats on the floodplain of the Murrumbidgee River 20 km west of Gundagai, NSW. All loose materials within the quadrat were collected, which included a mixture of soil and organic matter; most of the organic material collected was dry, comprising decaying leaves and sticks, and some fresh grasses. The most common vegetation was river redgum (*Eucalyptus camaldulensis*) followed by casuarina (*Casuarina cunninghamiana*), weeping willow (*Salix babylonica*) and various grasses. All anthropogenic litter such as plastics and glass bottles was removed before bagging. The leachate was made using a similar technique to that of Mitrovic et al. (2014). Floodplain materials were placed in two 70-L bins and soaked in 100 L of reverse osmosis water for 2 weeks at 4°C in the dark. Floodplain materials were swapped in/out of the bins every 3 days to maximise leachate concentration. The resulting leachates were then filtered through a series of filter sizes (10 and 1.3 μm) to 0.5 μm using a vacuum pump and glass fibre filter papers. Once filtration was complete the leachate was homogenised and frozen at −20°C. The final $\delta^{13}C$ signature of the homogenised leachate was −27.50‰.

2.3 | Study design

The experiment ran for 34 days from 31 October until 4 December 2019 with water temperatures ranging from 18 to 24°C during the day. There was no significant difference in water temperatures across treatments throughout the entire study. Mesocosm bags were filled one day before commencing the experiment using an electric pump and hose with a 4-cm aperture. A flow rate was calculated for the pump and the filling of each bag was timed to ensure an equal volume of water in each mesocosm. Day (D0) samples were taken after all bags were filled and before the leachate was added. The leachate was then added on 31 October (referred to as D0). Leachates were added in three concentrations determined via the dissolved organic carbon (DOC) content of the leachate: low (1 mg C/L), medium (4 mg C/L) and High (8 mg C/L) all performed in triplicate including a control (no leachate addition). The carbon:nitrogen:phosphorus (C:N:P)

ratio of leachate based on nitrogen oxides (NOx) and soluble reactive phosphorus (SRP) was 279:17:01. These additions were chosen to represent three different sized flow events and the consequent levels of allochthonous inputs from a small "fresh" up until an overbank flood, based off data taken from previous studies in Australian rivers (Nielsen et al., 2016; Westhorpe & Mitrovic, 2012). The biotic and chemical parameters within the mesocosms closely resembled those of lowland rivers. However, a key limitation in this study is the inability to reproduce the turbulence and flow velocity of a flowing river, which may affect zooplankton feeding behaviours and phytoplankton competition. Thus, these mesocosms do not fully represent the food-web dynamics of a lotic system.

2.4 | Field sampling and analysis

In order to measure changes in the balance between photosynthesis and respiration, dissolved oxygen and temperature were measured using a HACH HQ20 LDO probe. Measurements were taken between 10:00 and 11:00 hrs on D0, D1, D3, D5, D6, D12, D20, D27 and D34. Samples of nutrients and chlorophyll-*a* (Chl-*a*) were collected on D0, D1, D4, D8, D20 and D34. DOC, SRP and NOx samples were collected in pre-washed and sample rinsed 250-ml PET bottles, filtered to 0.45 µm using cellulose acetate syringe filters and then frozen. Samples were analysed using a segmented flow analyser (OI Analytical Model FS3100) according to standard methods (APHA, 2005). Samples for Chl-*a* were determined by filtering 250 ml of water onto GF/C filters. Filters were frozen until subsequent determination by boiling ethanol extraction according to Standard Methods (APHA, 2005). Half of each Chl-*a* filter paper was removed before analysis and stored for $\delta^{13}C$ isotope analysis of particulate organic matter (POM). Filter papers for $\delta^{13}C$ isotope analysis were dried at 60°C for 48 hr, sealed in sterile bottles and analysed at Griffith University where a standard hole punch was taken from each paper for analysis to guarantee consistent weights. Samples were analysed using a continuous flow isotope mass spectrometer (GV Isoprime Eurovector EA 3000). Results were determined using IAEA-CH-6 as standard reference material.

Composite water column samples for phytoplankton (autotrophic and mixotrophic algae) and microzooplankton (amoebae and ciliates) were taken using a bendable 1-m-long (4-cm aperture) plastic pipe, with samples (250 ml) preserved using 3 ml of Lugol's iodine solution. Samples were taken on D0, D1, D2, D4, D6, D8, D12, D20, D27 and D34 and counted at ×200 magnification on a compound microscope using Sedgwick rafter counting cells. Measurements for biovolume of algae were taken using an Olympus DP72 camera and cellSens Standard software (version 1.3). Twenty individual cells of each species were measured to achieve a reliable average. Algae were identified using the keys of Prescott (1978) and Entwisle et al. (1997). Amoebae and ciliates were counted with phytoplankton samples and identified using the key of Patterson (1996). Phytoplankton were separated into broad functional groups (Chlorophytes, Cyanophytes, Diatoms and Mixotrophs) similar to those of Karlsson et al. (2007). Mixotrophs were defined as anything previously shown to exhibit mixotrophic behaviour as well as any ciliates or amoeba which were considered potentially mixotrophic. For detailed information on phytoplankton classification see Table S1.

Samples of zooplankton (Copepods and Cladocera) were collected for enumeration and stable isotope analysis on D0, D1, D4, D8, D12, D20, D27 and D34. For each sample, 10 L of mesocosm water was passed through a 53-µm mesh and decanted into a pre-rinsed PET bottle. Zooplankton were purged for 4 hr, using 0.5-µm filtered mesocosm water, then preserved with >70% ethanol v/v. For enumeration, zooplankton were concentrated to ×250 and a subsample (25% of total sample) counted on a Sedgwick rafter cell at ×100 magnification using a compound microscope. Zooplankton were identified using the key of Shiel (1995). Samples were returned to bottles and preserved using the original ethanol from each bottle. Zooplankton samples for $\delta^{13}C$ analysis were rinsed thoroughly three times with reverse osmosis water to ensure that all ethanol was removed. Samples then were picked using a dissecting microscope at ×8 magnification. Zooplankton were sorted into appropriate taxa, then further cleaned for impurities such as organic matter, incorrect zooplankton group or filamentous algae, before being placed into 5-mm silver capsules. Samples were acidified using 1 mol/L hydrochloric acid (HCl) to remove any inorganic carbon from samples and dried at 60°C for 24 hr. Zooplankton samples were pooled between replicates to ensure that there was sufficient biomass for analysis. Samples were analysed at Griffith University using a continuous flow isotope mass spectrometer (GV Isoprime Eurovector EA 3000). Results were determined using IAEA-CH-6 as standard reference material.

2.5 | Data analysis

Permutational multivariate analysis of variance using PRIMER 6.0 + PERMANOVA (Anderson et al., 2008) was used to analyse data for significant differences between treatments. Pairwise comparisons within PERMANOVA were used to test for significant differences between treatments within sampling days, similar to the method of Hitchcock et al. (2016). All data were checked for normal distribution and homogeneity of variance using PERMDISP and draftman's plots. Euclidean distances were used for environmental variables and transformed using $\log_{10}$ to account for skewed distribution and then normalised. Bray–Curtis distances were used for phytoplankton (autotrophs and mixotrophs) and zooplankton, analyses were run separately for individual taxa or functional groups. Species data were transformed using a square-root transformation. As zooplankton $\delta^{13}C$ data was pooled, no multivariate analysis was conducted on the results. Instead, linear regressions were used to compare POM $\delta^{13}C$ signatures to zooplankton $\delta^{13}C$ and the ratio of mixotroph to autotroph biovolume using SIGMAPLOT software. All regressions were checked for normal distribution using Shapiro–Wilk's test. The ratio of mixotrophs to autotrophs was calculated using the total biovolume of potential mixotrophs versus the total

biovolume of all obligate autotrophic algae. These ratio data were then $\log_{10}$-transformed to account for skewness.

3 | RESULTS

Dissolved organic carbon and nutrient concentrations (Figure 1a–c) increased relative to leachate additions and returned to starting concentrations by D27; however, on D20 NOx concentrations increased sharply in all leachate additions with the low and medium treatments both reaching >100 μg/L whereas the high treatment reached >60 μg/L and the control remained unchanged. SRP concentrations in the high tDOM treatment remained higher than all other treatments for the duration of the experiment. Dissolved oxygen (DO; Figure 1d) appeared inversely related to leachate additions, showing the largest decrease immediately after leachate addition on D1 in the high DOM treatment (20%). DO did not return to levels similar to the control until D20. Chl-*a* concentrations in the leachate additions were significantly different from the control until D34 (Figure 1e). During the first nine days Chl-*a* increased in the control and the low and medium tDOM treatments with Chl-*a* significantly higher in the control. By contrast, Chl-*a* decreased in the high tDOM treatment during the first nine days. Chl-*a* concentrations in the medium and low treatments were not significantly different throughout the study. By D20, Chl-*a* had decreased sharply in the control treatment, falling to a level below that of all other treatments. Measures of Chl-*a* on D34 revealed similar levels across all treatments at the end of the experiment.

Chlorophytes and mixotrophs were the most abundant algal groups throughout the experiment and at times were one to two orders of magnitude higher in biovolume than diatoms and cyanophytes (Figure 2). The biovolume of all algal groups differed significantly across time and the time × treatment interaction ($p = 0.001$, all groups; Table 1), yet only mixotroph and cyanophyte biovolume was significantly different between treatments ($p = 0.001$; Table 1). Chlorophyte and diatom biovolume were not significantly different between treatments ($p > 0.05$) with chlorophyte biovolume (Figure 2a) generally increasing in all treatments from D0 to D34. Mixotroph biovolume (Figure 2d) increased in all leachate treatments immediately after carbon additions, peaking in the high treatment at 7.4 mm^3/L on D12, which was 6.0 mm^3/L higher than the control (1.3 mm^3/L) and 2.2 mm^3/L higher than the medium and low treatments, which were not significantly different from each other ($p > 0.05$). Mixotroph biovolume was higher than all other algal

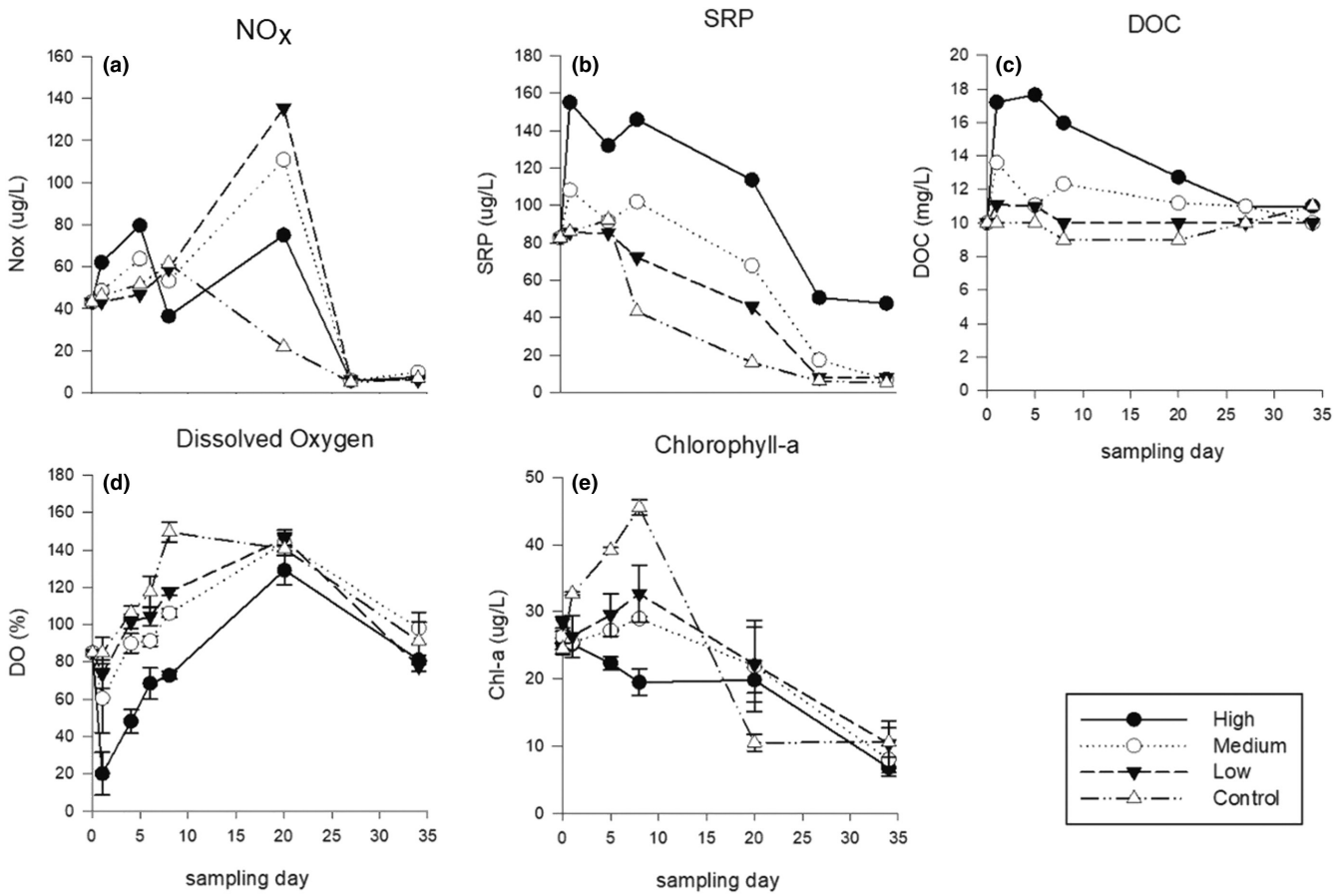

FIGURE 1 Nitrogen oxides (μg/L), soluble reactive phosphorus (SRP, μg/L), dissolved organic carbon (DOC, mg/L) dissolved oxygen (DO, %) and chlorophyll-*a* (Chl-*a*, μg/L), in all treatments across the sampling period. Treatments are identified by black circles (high), white circles (medium), black triangles (low) and white triangles (control). Error bars represent *SEM*

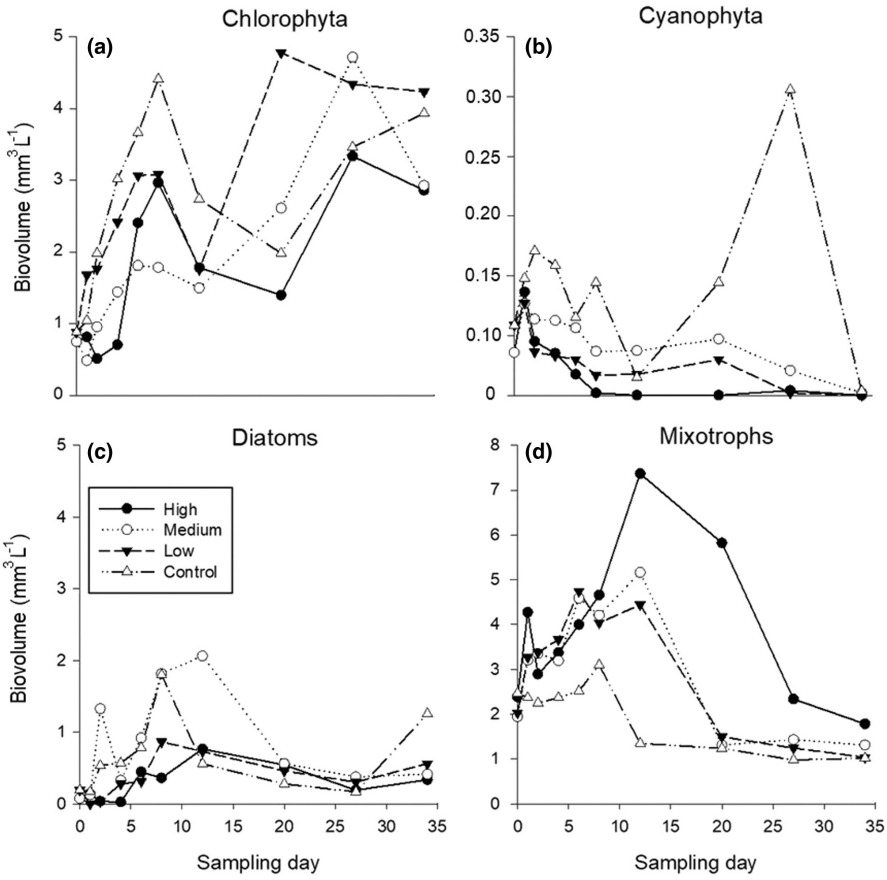

FIGURE 2 Total biovolume (mm^3/L) of algal groups (a, chlotophytes; b, cyanophytes; c, diatoms; d, mixotrophs) in each treatment over time. Treatments are shown by black circles (high), white circles (medium), black triangles (low) and white triangles (control). *SE* bars removed for clarity

groups within the medium and low treatments until D12 and in the high treatment until D20. Mixotroph biovolume in the high treatment was significantly different from the control by D4 ($p = 0.014$) and from medium ($p = 0.018$) and low ($p = 0.010$) treatments by D12. The low and medium treatments were significantly different from control by D4 (low; $p = 0.033$) and D6 (medium; $p = 0.009$) and returned to control levels on D20 ($p > 0.05$). The medium and low treatments were not significantly different from each other at any time point ($p > 0.05$). Chlorophytes dominated the biovolume throughout the entire study in the control and following reductions in mixotrophs in the tDOM treatments after D20.

Cyanophyte biovolume (Figure 2b) was highest in the control group, peaking at $0.305\,mm^3/L$ on D27. Leachate addition treatments led to lower cyanobacterial biovolume, declining from D0 levels until not being detected in the high treatment at D8 and at D27 and D34 in the low and medium treatments, respectively. Diatom biovolume (Figure 2c) was not significantly different between treatments and remained low throughout the duration of the experiment. For a full list of algal taxa identified in this study, see Table S1.

The cell concentration of all mixotrophic genera and ciliates varied significantly through time ($p = 0.001$, all groups). *Trachelomonas*, *Cryptomonas*, *Chroomonas* and amoebae also were all significantly different between treatments ($p = 0.001$; Table 1) with leachate additions resulting in higher concentrations of mixotrophs compared to the control (Figure 3a–d). *Trachelomonas* concentrations (Figure 3a) immediately increased following leachate addition. Low and medium treatments were significantly different from the control from D1 to D12, peaking at 900 cells/ml (low) and 1,100 cells/ml (medium) on D6 and D12, before declining to levels similar to that of the control by D20. *Trachelomonas* in the high treatment peaked at 1,200 cells/ml from D8 to D20, over five-fold higher than concentration of the control. The high DOM treatment was significantly different from the control from D4 to D27 and from medium and low on D8 and D20–34. *Trachelomonas* concentrations in the control did not change from D0 levels until D12 when they decreased to 200 cells/ml from approximately 600 cells/ml.

Cryptomonas and *Chroomonas* (Figure 3b,c) followed a similar pattern to *Trachelomonas*, with very large peaks in concentration on D12 to D20 in the high treatment ($p < 0.01$, compared to that of the control) and D8 to D12 in the medium and low treatments ($p < 0.01$, compared to that of the control). The high treatment was significantly different from medium and low treatments for *Cryptomonas* and *Chroomonas* from D4 to D20 ($p < 0.05$). Like *Trachelomonas*, medium and low treatments were at similar levels to those of the control by D20, and by D27 all treatments were at a similar concentration.

The amoeba community was composed entirely of individuals from the *Saccamoeba* genus. Amoeba concentrations (Figure 3d) were significantly higher in all leachate treatments versus that of the control ($p = 0.001$). Amoeba concentrations increased in all leachate treatments until D12 after which concentrations in the high treatment remained between 80 and 100 cells/ml, whereas medium and low treatments dropped to much lower concentrations (<40 cells/

TABLE 1 PERMANOVA main test results for differences between treatments.

	Between treatments			Between days			Days × treatment		
Group	***df***	**Pseudo-*f***	***p***	***df***	**Pseudo-*f***	***p***	***df***	**Pseudo-*f***	***p***
Functional groups									
Chlorophyceae	3	32.95	>0.05	9	163.39	0.001	27	15.32	0.001
Mixotrophs	3	100.87	0.001	9	98.71	0.001	27	12.78	0.001
Diatoms	3	20.64	>0.05	9	27.94	0.001	27	10.45	0.001
Cyanophyta	3	11.889	0.001	9	10.7	0.001	27	4.87	0.001
Mixotroph groups									
Trachelomonas	3	81.48	0.001	9	105.64	0.001	27	10.27	0.001
Cryptomonas	3	5.01	0.001	9	12.92	0.001	27	3.34	0.001
Croomonas	3	5.46	0.001	9	5.46	0.001	27	2.59	0.001
Amoeba	3	7.17	0.001	9	3.48	0.001	27	1.23	0.001
Ciliates	3	1.96	>0.05	9	7.01	0.001	27	1.19	0.001
Zooplankton									
Daphnia	3	28.32	0.001	6	68.84	0.001	18	9.07	0.001
Cyclopoid copepods	3	12.10	0.001	6	40.15	0.001	18	3.10	0.001
Calanoid copepods	3	14.59	0.001	6	117.89	0.001	18	5.90	0.001
Nauplii	3	12.20	0.001	6	679.31	0.001	18	7.86	0.001
Bosmina	3	11.56	0.001	6	83.15	0.001	18	5.97	0.001
Ceriodaphnia	3	6.06	>0.05	6	65.42	0.001	18	2.39	0.001
Copepodites	3	16.78	0.001	6	401.5	0.001	18	11.87	0.001

ml) by D20. Amoeba concentrations in the high treatment were significantly different to that of the control from D2 until the end of the experiment ($p < 0.05$). Medium and low treatment amoeba concentrations were significantly different to that of the control from D4 until D27. Ciliates (Figure 3e) were composed primarily of oligotrich ciliates that were not significantly different between treatments ($p > 0.05$) but were significantly different across time ($p < 0.05$).

All zooplankton groups excluding *Ceriodaphnia* (Figure 4), responded significantly across treatments ($p = 0.001$) and time ($p = 0.001$), with the effect of treatments varying strongly through time. All leachate additions led to significantly higher abundances of nauplii, copepodites, cyclopoid copepods and *Daphnia* compared to the control. In contrast, calanoid copepod abundance was highest in the control and low treatments, and *Ceriodaphnia* concentrations were not consistently different between control and leachate additions.

Nauplii and copepodite concentrations (Figure 4a,b) followed similar patterns to each other, increasing sharply between D1 and D5. Nauplii peaked in all leachate treatments on D12 (800–1,200 ind/L) and were two- to three-fold higher than that of the control (400 ind/L). Nauplii concentrations in all leachate treatments were significantly different from that of the control from D5 to D20 ($p < 0.01$). Copepodites also peaked on D12 in the medium (375 ind/L) and low (250 ind/L) treatments, with the high treatment peaking later on D20, at a 10-fold higher concentration (325 ind/L) than the control. Copepodite concentrations were significantly different between the control and the high treatment on D5 and D20, the medium treatment on D5–34 and the low treatment on D12–20. By D27, both nauplii and copepodite concentrations had reduced to levels close to that of the control.

Cyclopoid abundance (Figure 4c) increased until D12 where it peaked in the control (100 ind/L), low (150 ind/L) and medium (220 ind/L) treatments. Cyclopoid concentrations were significantly different between the control and the medium treatment from D1 to D27, and the low treatment on D1, D12 and D27. After D20, concentrations in the high treatment rose sharply to >400 ind/L until D27, after which they declined to a level similar to those observed in all treatments. Cyclopoid concentrations were significantly different from that of the control in the high treatment on D5–27, in the medium on D1–27 and in the low treatment on D1, D12 and D27. Calanoid abundance (Figure 4d) increased over time yet was generally an order of magnitude lower than cyclopoid copepods in all treatments. The control and low leachate treatments consistently supported the highest calanoid abundance throughout the study, peaking in concentration on D27 (low: 48 ind/L; Control: 39 ind/L) before reducing to the same levels as all treatments on D34. The high leachate treatment always supported the lowest abundance of calanoid copepods and was significantly different from the medium and low treatments on D12–27 and from the control on D20–27.

Daphnia concentrations (Figure 4e) were significantly different between leachate additions and the control on D1, D5 and D20 ($p < 0.05$). All leachate treatments had similar abundances until D12

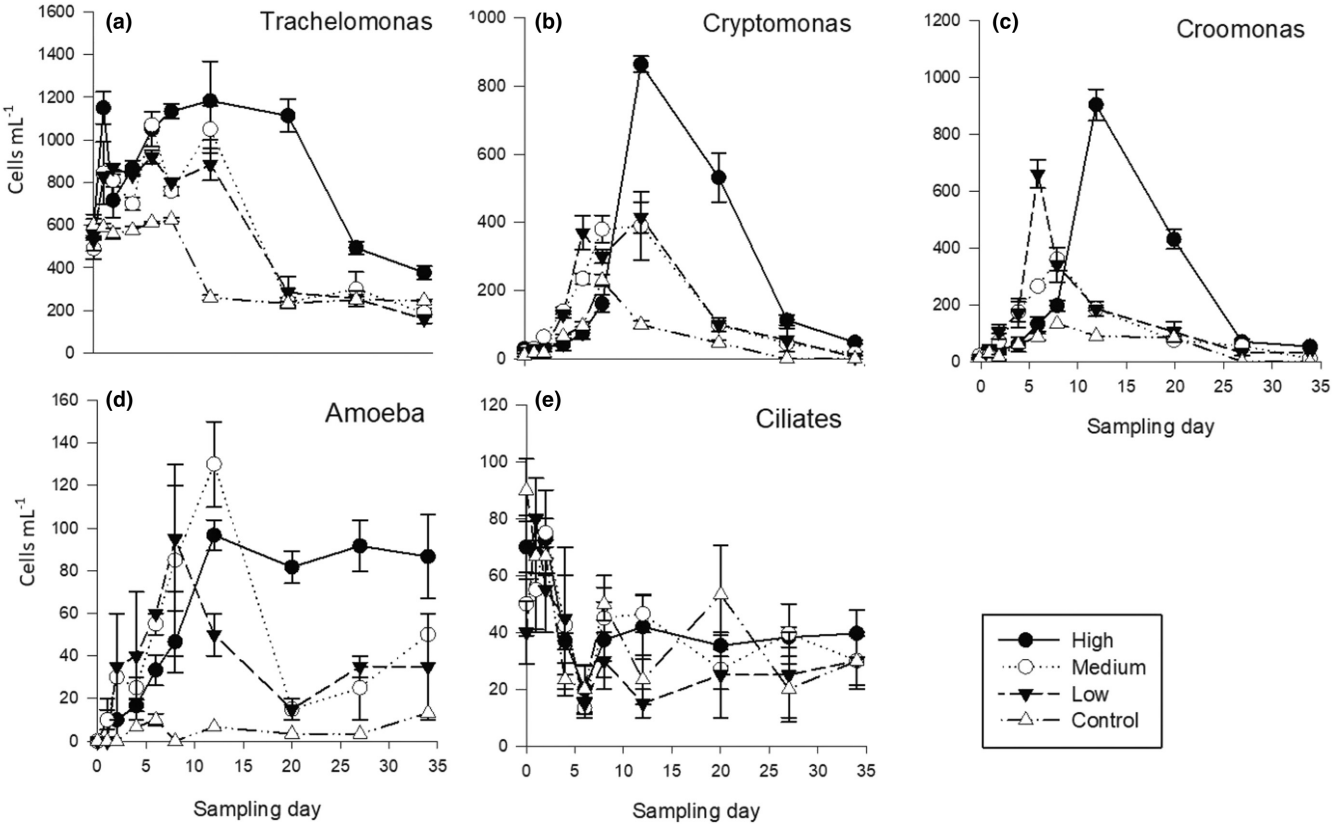

FIGURE 3 Mean concentration (cells/ml) of mixotrophic algae, amoeba and ciliates in each treatment over time with *SEM*. Treatments are indicated by black circles (high), white circles (medium), black triangles (low) and white triangles (control)

when the low (180 ind/L) and medium (175 ind/L) treatments peaked at levels double that of the control (85 ind/L). From D20 onwards, *Daphnia* concentrations in the low and medium treatments were not significantly different from the control ($p > 0.05$). The high treatment continued to increase, leading to a peak in concentrations (220 ind/L; $p = 0.001$) on D20 of approximately 10-fold that of all other treatments. *Daphnia* concentrations in the high treatment then declined until all treatments were at similar levels by D34 and were not significantly different. *Ceriodaphnia* in all treatments followed a similar pattern, increasing to peak concentrations on D12 with the highest concentrations in the control (>100 ind/L) and significantly different from all other treatments ($p < 0.05$).

The POM $\delta^{13}C$ signatures (Figure 5a) changed immediately after leachate additions with the high leachate treatment dropping to −31.5‰ until D12; the medium treatment also briefly dropped to this level on D1. POM $\delta^{13}C$ signatures showed a clear pattern reducing inversely to leachate additions, with this pattern maintained for the entire study. POM $\delta^{13}C$ signatures increased sharply from D5 to D12 in the control treatment, and in all leachate addition treatments from D12 to D20. On D12 the largest difference between POM signatures occurred when the high treatment (−32‰) was >8‰ lower than the control (−24‰).

Zooplankton $\delta^{13}C$ signatures (Figure 5b–d) followed very similar patterns across all treatments, with signatures starting low, before increasing and peaking around D20. Between D0 and D5, $\delta^{13}C$ signatures decreased in all zooplankton groups and treatments, excluding a 1‰ increase between D0 and D1 in *Daphnia* and *Ceriodaphnia* in the high treatment. After D5, zooplankton in the high leachate treatment had the most depleted $\delta^{13}C$ signature and remained closer to the leachate signature (−27.5‰) than any other treatment. By contrast, all measured zooplankton groups in the control had the most enriched $\delta^{13}C$ signatures at each time point and were furthest from the leachate $\delta^{13}C$ signature. As with the POM, zooplankton $\delta^{13}C$ signatures appeared inversely related to leachate additions, with more depleted $\delta^{13}C$ values in the treatments with the highest amount of leachate added. By D12 cyclopoid $\delta^{13}C$ values had begun to clearly differentiate between the high carbon and control treatments, leading to the largest differences between treatments (>6‰) on D27 with $\delta^{13}C$ signatures in the control at −18‰ and in the high treatment at −24‰. *Daphnia* and *Ceriodaphnia* $\delta^{13}C$ signatures (Figure 5c,d) followed very similar trends to cyclopoid copepods although they started with a more depleted signature at −27.9‰. In a similar way to cyclopoid copepods, *Daphnia* $\delta^{13}C$ values diverged after D5 with the biggest difference falling on D12 when signatures in the high leachate treatment were −28.2‰ compared to −21.8‰ for the control.

Regression analysis found that the ratio of mixotroph to obligate autotroph biovolume (Figure 6a) correlated strongly with POM $\delta^{13}C$ signatures ($R^2 = 0.62$, $p < 0.0001$). A biovolume ratio >0 (indicating higher total mixotrophic biovolume than autotrophic) correlated

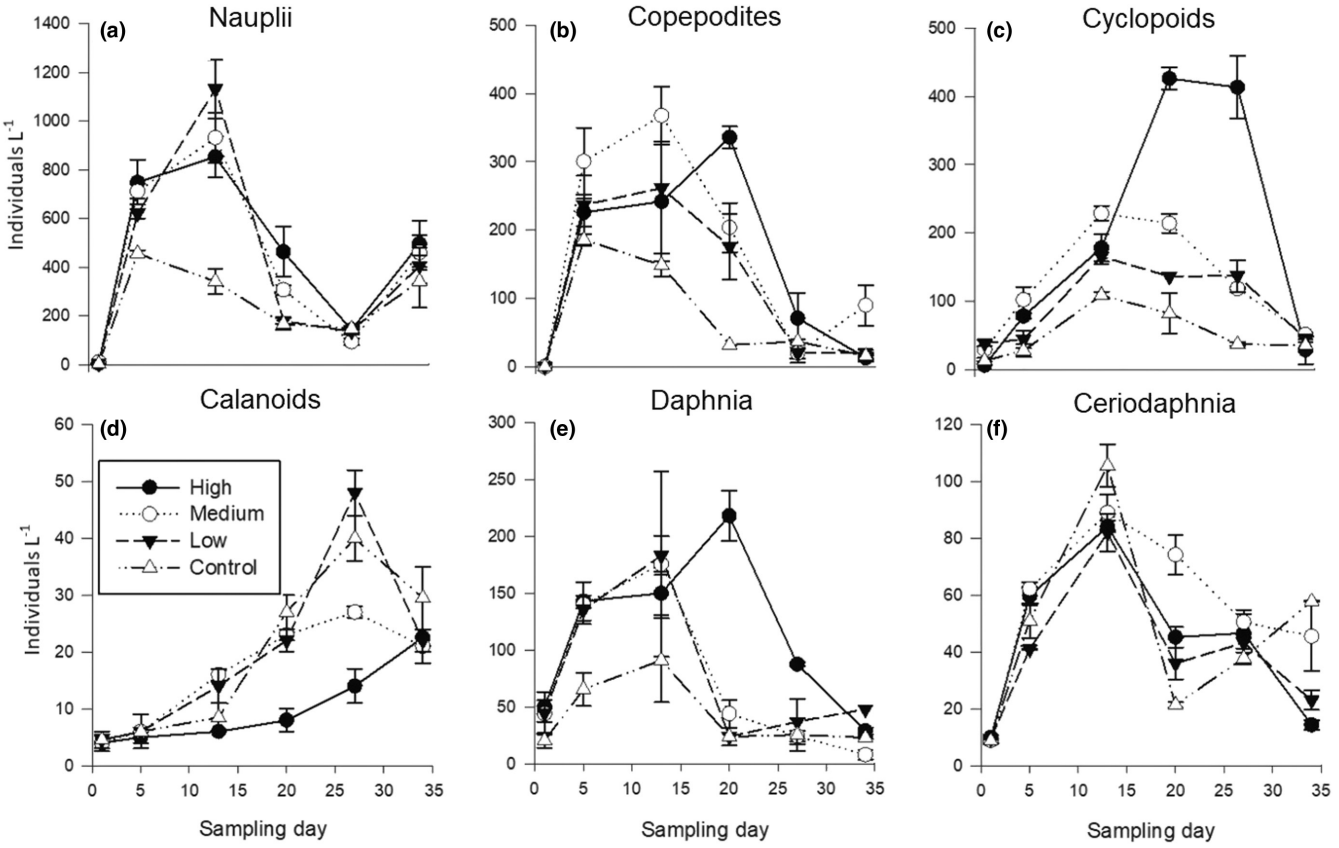

FIGURE 4 Mean zooplankton concentrations for each zooplankton group and treatment. Error bars indicate *SEM*

with more depleted POM $\delta^{13}C$ signatures (<−28‰) compared to those when autotrophic algae were more abundant than mixotrophs (<0) when POM $\delta^{13}C$ signatures were >−28‰.

Total zooplankton $\delta^{13}C$ signatures correlated strongly with POM signatures (R^2 = 0.71, $p < 0.0001$; Figure 6b). Regression analysis found that cyclopoid copepods in the high leachate treatment were strongly correlated to POM signatures ($R^2 = 0.82$, $p < 0.0001$), whereas cyclopoid copepods in the control treatment were not significantly related to POM ($p > 0.05$). *Daphnia* and *Ceriodaphnia* $\delta^{13}C$ signatures in all treatments were significantly correlated to POM signatures ($p < 0.0001$; $R^2 > 0.85$ all treatments).

4 | DISCUSSION

This study aimed to understand how allochthonous organic matter can influence riverine food-web structure and production. Our experimental results expanded on those of previous similar mesocosm studies (Hitchcock et al., 2016; Karlsson et al., 2007; Mitrovic et al., 2014) and provide strong evidence for the important role of high quality allochthonous sources of carbon in aquatic food webs. In terms of exploring a response to inputs of organic carbon, all additions of tDOM resulted in significant increases in mixotrophic flagellates and the abundance of most measured zooplankton (nauplii, copepodites, cyclopoid copepods, *Daphnia*) compared to the control. Further, declines in concentrations of mixotrophs, cyclopoid copepods and *Daphnia* towards the end of the experiment, and simultaneous increases in chlorophytes and calanoid copepods indicate changes away from allochthonous support as resources diminished over time. These results support our first hypothesis that allochthonous inputs can significantly boost production in the lower food webs of aquatic ecosystems.

The $\delta^{13}C$ values of zooplankton and POM in the high leachate treatment were closer to the range of the leachate $\delta^{13}C$ values than any other treatment, reflecting both the influence of tDOM additions on the POM pool and the use of tDOM by higher trophic levels, supporting our second hypothesis. However, the expected reduction in phytoplankton biovolume as a response to leachate inputs was not as clear as expected, with only Chl-*a* and cyanobacteria clearly decreasing, and chlorophytes and diatoms showing no significant difference in biovolume across treatments. Instead, mixotrophs increased complimentarily to autotrophic production, dominating the algal biovolume in tDOM amendments. This resulted in large net increases to phytoplankton biovolume and potentially played a large role in driving changes in zooplankton growth by providing a trophic link between allochthonous carbon and primary consumers. Mixotroph biovolume also was significantly related to changes in POM $\delta^{13}C$ values, further indicating that they were a potentially important path for tDOM uptake.

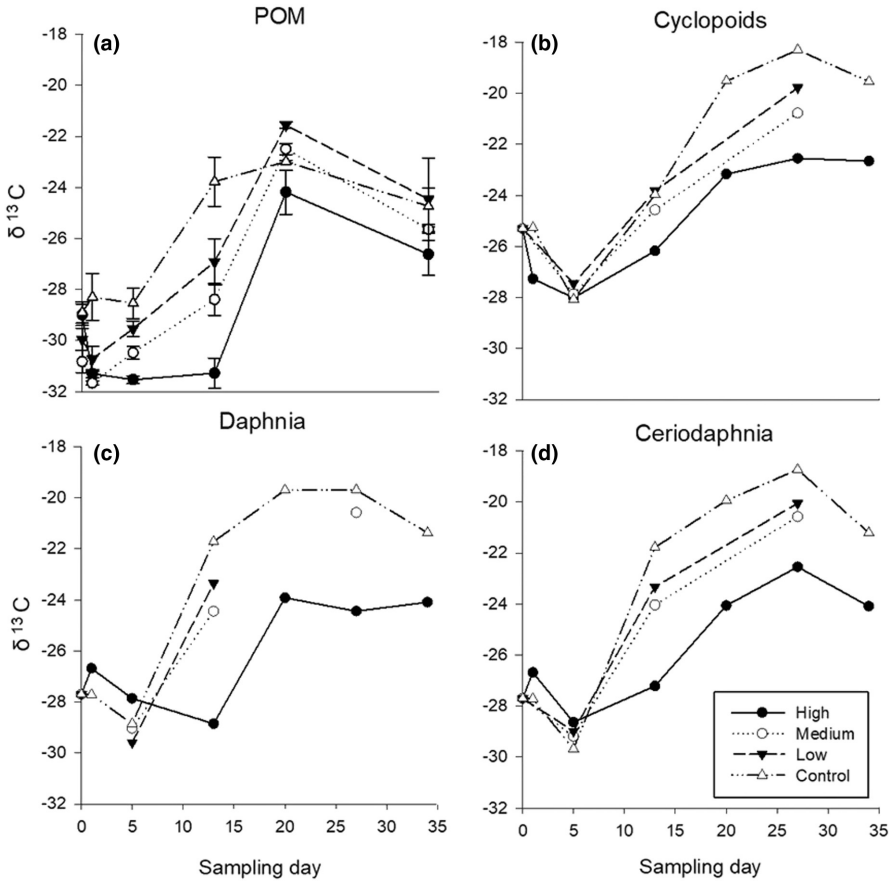

FIGURE 5 $\delta^{13}C$ signatures of cyclopoid copepods, Daphnia, Ceriodaphnia and particulate organic matter (POM). All zooplankton samples were pooled between replicates. Error bars on POM indicate *SEM*. Samples were not analysed where values are missing for medium and low treatments

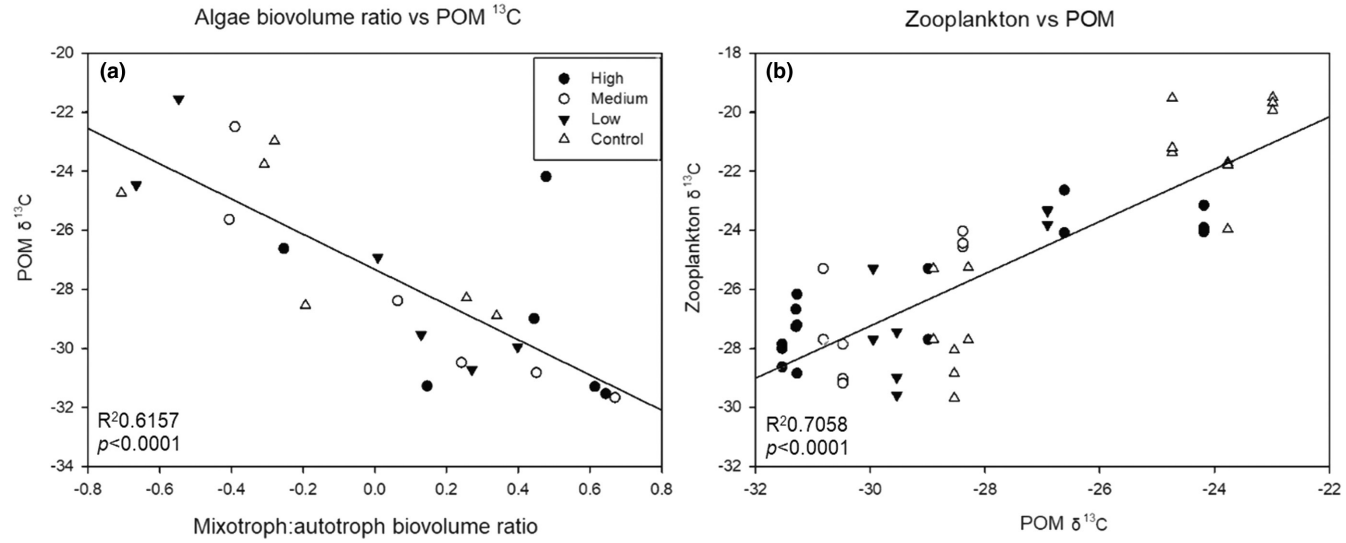

FIGURE 6 Linear regression data from all sampling days for: (a) biovolume ratio of mixotrophic:autotrophic phytoplankton (log_{10}-transformed) versus the $\delta^{13}C$ signature of particulate organic matter (POM); and (b) POM $\delta^{13}C$ signature versus zooplankton $\delta^{13}C$ signature (all taxa). Data are separated into treatments: high (black circles), medium (white circles), low (black triangles) and control (white triangles)

4.1 | Phytoplankton and mixotroph responses

Changes in the phytoplankton community after the addition of tDOM offered two insights into the role of allochthonous carbon in phytoplankton dynamics. Firstly, mixotrophic algae can comprise the largest portion of the algae population and dominate the algal community following pulses of organic matter. Secondly, obligate autotrophs may respond more dynamically to allochthonous inputs than previously suggested, as nutrients from tDOM support phytoplankton growth simultaneously to heterotrophic production. Our findings showed tDOM pulses can lead to large increases in heterotrophic production and, subsequently, also can stimulate

autotrophic production, potentially leading to a range of available resources for consumers. These findings are similar to those of Faithfull et al. (2011) who found that autotrophic production was unaffected by glucose addition and did not reduce when mixotrophic algae dominated the community. This contrasts with previous studies showing that bacterioplankton out-competed obligate autotrophs for nutrients in the presence of an allochthonous carbon source and significantly reduced the biomass of phytoplankton (Carney et al., 2016). The differences in results between this and previous studies may be a result of variation in the existing nutrient pool. Hitchcock and Mitrovic (2013) previously have shown that additions of DOM in nutrient-poor systems may lead to phytoplankton nutrient limitation as a consequence of bacterial uptake of phosphorus, but this was not observed in systems with higher initial nutrient concentrations. In this study there were high concentrations of NOx and SRP both in the leachate and within the ambient water column. This is likely to have meant that despite a higher proportion of carbon in the treatments compared to nutrients, potentially there were sufficient nutrients for both heterotrophs and autotrophs during the first weeks of the experiment. The anomalous peak in NOx concentrations on D20 may be an artefact of the differences in phytoplankton and bacterial community structures within treatments at that time, which potentially influenced the processing of nitrogen waste products, yet without in-depth bacterial community data, this is entirely speculative.

We unfortunately do not have data for bacterial production in this study; however, DO concentrations within the first week provide some insight into bacterial respiration and potential production. Changes in DO between treatments were approximately proportional to differences in tDOM additions with the lowest DO concentrations occurring in the high tDOM treatment in the first 24 hr. These rapid reductions in DO are consistent with patterns of bacterial carbon consumption of labile portions of the DOM pool (Hitchcock & Mitrovic, 2015). We hypothesise that bacterial production was likely to have been the energetic pathway linking tDOM additions and mixotrophs in this study.

Mixotrophs were the dominant functional group (by biovolume) in all tDOM additions until D12 (low, medium treatments) and D20 (high treatment), whereas chlorophytes dominated the algal biomass in the control treatment for the entire study. Blooms in *Trachelomonas* and later *Cryptomonad* populations in the tDOM treatments may have been a major resource supporting the increases in zooplankton populations. Previous studies have found mixotrophs to be a higher quality food source for zooplankton relative to the bacteria which they consume (Jäger et al., 2014; Katechakis et al., 2005) and may be preferentially preyed upon by some zooplankton taxa (Hansson et al., 2019). In particular, *Cryptomonas* has been found to substantially improve Daphnia growth even when in low concentrations (Brett et al., 2009). Our data support previous studies suggesting that mixotrophic flagellates play a stabilising role between autotrophic and heterotrophic production and food quality (Flynn et al., 2013; Worden et al., 2015), and may play an important role in mobilising allochthonous carbon for higher trophic levels (Jäger et al., 2014).

Mixotrophy appeared to play an important role in driving the POM $\delta^{13}C$ signature throughout the study. As the ratio of mixotrophic to autotrophic biovolume increased, $\delta^{13}C$ signatures of the POM were typically more depleted and closer to the leachate signature ($R^2 = 0.62$). This suggests that mixotrophs were using tDOM as an energy or nutrient source which resulted in POM signatures reflecting the leachate additions. Furthermore, the POM $\delta^{13}C$ signature was strongly correlated to signatures of zooplankton. This is consistent with previous findings in Patagonian lakes where mixotrophy was a major driver of the $\delta^{13}C$ signature of POM and calanoid signatures in the system (Trochine et al., 2015). Without knowing the initial $\delta^{13}C$ values of mixotrophic algae, relationships between leachate uptake and mixotrophic biovolume are based entirely on correlations and thus, are somewhat speculative. However, this relationship between mixotrophy, POM and zooplankton suggests that mixotrophy may play a significant role in linking allochthonous DOM and bacteria to higher trophic levels.

In the larger protists, only amoeba concentrations increased with tDOM additions with ciliate concentrations showing no significant difference between treatments. This may be a consequence of intensive zooplankton predation, as zooplankton can exert strong top-down pressure on ciliate populations (Sommer & Sommer, 2006). In a similar mesocosm study, Degerman et al. (2018) found that zooplankton predation greatly reduced ciliate populations despite abundant food (bacteria and nanoflagellates) for growth. In our study ciliate abundance declined from D1 in all treatments whereas zooplankton abundance simultaneously increased, potentially intensifying predation pressure to a point where any increases in ciliate abundance were unable to be observed.

The effects of our leachate additions on light attenuation within the mesocosms were not measured, yet there is potential for it to have affected our results to some degree, particularly within the phytoplankton community. Despite obligate autotrophs showing no significant difference across treatments, Chl-*a* concentrations were clearly lower in all tDOM treatments compared to the control with the high tDOM addition having by far the lowest Chl-*a* concentrations. Previous research has found coloured DOM to have a significant impact on the wavelengths that coincide with chlorophyll pigments and, thus, photosynthesis (Kirk, 1976). Likewsie, in boreal lakes DOC and coloured DOM concentration has been found to increase light attenuation and suppress photosynthesis (Karlsson et al., 2009; Thrane et al., 2014). Reduced light intensitities may increase the competitive advantage of mixotrophs which previously have been found to dominate phytoplankton communities during periods of low light (Wilken et al., 2017). Unfortunately, without direct measurements of light it is difficult to quantify the potential impact that any light intentsity decreases had on the phytoplankton community. However, based on the similar biovolume of obligate autotrophs across all treatments in this study, we argue that light attenuation was not the driving factor in increasing mixotrophic biovolume.

4.2 | Zooplankton responses to tDOM

Terrestrial DOM additions had a clear effect on the zooplankton community, similar to those found in other studies in south-east Australia (Hitchcock et al., 2016; Mitrovic et al., 2014) and Europe (Degerman et al., 2018; Faithfull et al., 2011). Additions of tDOM resulted in large increases in total zooplankton abundance, of which *Daphnia* and Cyclopoida spp. were the most pronounced. *Daphnia* growth and reproduction using tDOM via bacteria and mixotrophic flagellate intermediaries has been found to be equal to or better than when compared to completely autotrophic diets (Hiltunen et al., 2017; Jäger et al., 2014; McMeans et al., 2015). Evidence of allochthony in cyclopoid copepods has been found in Swedish lakes (Berggren et al., 2014) and Australian river mesocosm experiments (Mitrovic et al., 2014), and previous studies suggest that cyclopoid production may be limited by tDOM as a result of their reliance on microbial food chains as a food source (Berggren, Laudon, et al., 2010; Berggren, Ström, et al., 2010; Jürgens & Jeppesen, 2000). *Daphnia* and cyclopoid copepods reflect two distinctly different feeding behaviours for the consumption of allochthonous DOM (Berggren et al., 2014). As filter feeders, *Daphnia* feed directly on bacteria and algae, removing several food chain links from the microbial loop, making the uptake of allochthonous DOM more efficient (Hiltunen et al., 2017; Jäger et al., 2014). By contrast, raptorial cyclopoid copepods use allochthonous DOM through consuming microzooplankton (rotifers/ciliates) at the top of the DOM–bacteria–nanoflagellate microbial pathway (Jürgens & Jeppesen, 2000; Karlsson et al., 2003; Pace et al., 2004). These different feeding strategies affect the efficiency of carbon transport to higher trophic levels as cyclopoid copepods require extra links in the food web compared to *Daphnia* (Jansson et al., 2007; Karlsson et al., 2007). Other filter feeders/bacterivores such as copepod nauplii also increased immediately after the tDOM additions. As a result, a broad successional change was evident following leachate addition. Filter feeders able to immediately consume bacterioplankton (*Daphnia* and copepod nauplii) peaked early, followed later by dominance of raptorial omnivores which require higher level primary consumers (ciliates, rotifers, small microcrustaceans) to access tDOM via consumptive pathways. This successional change in zooplankton also was evident in the $\delta^{13}C$ values of *Daphnia* and cyclopoid copepods in the high tDOM treatment as *Daphnia* diverged from the control and moved towards the leachate signature earlier than the cyclopoid copepods. These successional changes in the zooplankton community are broadly similar to those presented by Shabarova et al. (2021) who found that top-down food-web structure and resource limitation played an important role in defining eukaryotic dynamics in ponds and streams following large rain events.

The $\delta^{13}C$ signatures of zooplankton were relative to the proportion of leachate added, similar to the findings of Karlsson et al. (2007). Previous studies into zooplankton $\delta^{13}C$ have found that *Daphnia* and Cyclopoid copepods used allochthonous DOM as an important food source in their diets (Berggren et al., 2014). In this study, *Daphnia* $\delta^{13}C$ signatures had clearly shifted away from the control by D12 in the high treatment and were more depleted and much closer to the leachate $\delta^{13}C$ signature than all other treatments. *Daphnia* $\delta^{13}C$ values were significantly related to POM $\delta^{13}C$ values in all treatments; this is likely to have been a function of non-selective filter feeding, directly consuming bacteria, POM and mixotrophs supported by tDOM. Interestingly, *Ceriodaphnia*, which were not significantly different between treatments, showed a similar pattern in $\delta^{13}C$ values to *Daphnia*, with $\delta^{13}C$ values more depleted and closer to leachate values relative to the size of leachate additions. Cyclopoid copepods followed a similar trend in overall $\delta^{13}C$ values over time, however, were only significantly related to POM in the high leachate treatment. This may be an indicator of the different composition of POM between the high treatments and all others as cyclopoid copepods are selective raptorial feeders (Jürgens & Jeppesen, 2000).

In contrast to previous studies (Hitchcock et al., 2016; Karlsson et al., 2007), calanoid abundance appeared to be negatively correlated to carbon addition. This correlation may be highly species-dependent; however, increased abundances of cyclopoid copepods have been shown to exert top-down pressure through predation on calanoid adults and nauplii (Blumenshine & Hambright, 2003), as seen in the high and medium carbon treatments. Unfortunately, as a consequence of the low biomass of calanoid copepods throughout this study there was not the required biomass to perform stable isotope analysis. Consequently, it remains unclear if calanoid production was supported by the allochthonous DOM addition in this study.

4.3 | Ecological implications of allochthonous inputs

The energy pathways supporting the food web in these experiments were dynamic and changed quickly based on the pulses of allochthonous material added. Phytoplankton and zooplankton communities switched quickly between resources and zooplankton appeared able to use multiple trophic pathways for food resources. Ultimately, allochthonous resources appeared to support both autotrophic and heterotrophic production, and greatly increased zooplankton and net phytoplankton growth throughout the lower food web. These results have important implications for our understanding of river ecology and conceptualising how food webs respond to allochthonous tDOM pulses that commonly occur during floods and storm flows. From these data it is difficult to ascertain the exact relationship between the level of allochthonous tDOM input and increased mixotrophy and secondary production rates. Further studies targeting this question by measuring changes in production and consumption rates may prove useful in uncovering this relationship. Our results contrast with those of Shabarova et al. (2021) who found that organisms were washed out of the system following large rain events and took several weeks to re-establish despite nutrient loads increasing 100-fold. This is likely to have been a result of differences in flow

velocity and dilution, which although not present in our study, are an important consideration when comparing our results to real-world situations.

A core challenge in experimentally testing the effects of floodplain tDOM is recreating its bioavailability to ensure that results are environmentally relevant. Naturally leached tDOM contains a mixture of compounds of different labilities, with the majority often considered recalcitrant (Berggren, Laudon, et al., 2010; Berggren, Ström, et al., 2010). Despite this, most DOM addition studies have used glucose, assuming equivalence between glucose and natural tDOM in terms of ecological response. Recent studies, however, have pursued the use of more natural DOM additions. Hitchcock et al. (2016) showed that additions of leaf leachate may support increased zooplankton production, whilst Lefebure et al. (2013) used additions from soil leachate finding evidence of subsidy for copepods. The sources of organic matter that leach into flood waters may vary between soils and plant material; in the wetlands of the Murray River, Australia, where our study was conducted, the majority of organic matter leached following flooding is thought to derive from leaf litter (O'Connell et al., 2000; Robertson et al., 1999). Whitworth et al. (2012) found 60% of this floodplain tDOM was bioavailable after an extended dry period, which reduced to approximately 30% following subsequent inundation. The leachate used in this experiment was derived from a collection of floodplain material which included all loose soil and leaf litter. Whilst we did not specifically test the bioavailability of the leachate in this study there was a 27% reduction in DOC in the high treatment over the first 20 days compared to initial concentrations. This equates to a use of 61% of the tDOM leachate if we consider initial DOC in the water before additions to be recalcitrant. While filtering the leachate to 0.5-μm is likely to have increased its bioavailability by removing the need to process course particulate OM and woody debris, its effects still offer an accurate representation of what may occur in similar natural systems following floodplain inundation. Furthermore, nitrogen and phosphorus concentrations in the leachate were relatively high (indicating high quality) which is likely to have played a role in the strong response seen in the food web following additions. Interpreting these results, we can consider the leachate we used to be at the high end of lability compared to natural flood waters, although environmentally relevant for the ecosystem which we are testing. However, in systems where tDOM resources are of a lower quality (e.g., low nitrogen and phosphorus content) such as coniferous forests (Franke et al., 2013), it is possible that the impacts of tDOM additions would be less pronounced. Thus, as the quality of tDOM varies across ecosystems (Baldwin et al., 2016; Bunn et al., 2003), the effects of tDOM pulses on freshwater food webs also may vary accordingly.

Mixotrophy is a growing area of importance in river ecology (Flynn et al., 2013), and may be of particular relevance in environments where food-web production can switch quickly between allochthonous and autochthonous sources, such as lowland rivers. The ability to switch between major energy sources may be crucial for energy transfer. Indeed, mixotrophy creates smooth transitions between photosynthesis and heterotrophic production supporting food webs (Worden et al., 2015), and is likely to be a quantitatively significant link between allochthonous organic matter and higher consumers (Hansson et al., 2019). Furthermore, mixotrophy may increase the transfer efficiency of food webs by offsetting the carbon lost during respiration in higher trophic levels with photosynthesis and reducing C:N:P mismatch between producers and consumers (Katechakis et al., 2005; Ward & Follows, 2016). Emerging evidence also has shown that mixotrophs may be a more stable and nutritious food source for zooplankton than obligate autotrophs (Hansson et al., 2019). Mixotrophy may, therefore, greatly increase the transfer efficiency and quality of tDOM to zooplankton compared to energy transfer from the microbial loop alone (Flynn et al., 2013; Hiltunen et al., 2017). By fuelling mixotrophic growth while maintaining autotrophic production, allochthonous DOM may offer larger total food-web subsidies than thought previously. In terms of flow events increasing tDOM concentrations our data suggest that large pulses of tDOM can offer significant boosts to zooplankton production via both autochthonous and heterotrophic pathways. During these periods mixotrophs may be of particular importance for the transfer of terrestrial inputs to higher trophic levels and as a stable resource in highly variable environmental conditions.

5 | CONCLUSION

This study has expanded on the results of several previous mesocosm experiments using tDOM as an energy source for a freshwater food web. We found that mixotrophic algae play a major role in mobilising tDOM for higher trophic levels. Furthermore, a trophic succession of filter feeders changing to raptorial species was evident as a pathway for allochthonous energy transfer, as zooplankton able to exploit bacterial populations emerged following leachate additions and were then preyed upon by higher trophic levels such as copepods. Stable isotopes evidence showed that POM $\delta^{13}C$ signatures were correlated to the ratio of mixotroph versus obligate autotroph biovolume. POM and zooplankton $\delta^{13}C$ signatures were closely correlated, suggesting that mixotrophs played a major role in feeding zooplankton throughout the study. These results support our main hypothesis that pulses of allochthonous organic matter can significantly increase zooplankton growth. Furthermore, our results suggest that mixotrophy may be an important process for transfer of allochthonous energy to higher consumers in freshwater food webs. We contend that the use of allochthonous carbon in freshwater food webs is highly complex, with multiple potential pathways of energy transfer to higher trophic levels. Further research into the role of mixotrophic flagellates and their impact on consumers may greatly increase our underlying understanding of how allochthonous carbon is used by food webs.

AUTHOR CONTRIBUTION

Conceptualisation: MB, JH, SM, CB, DW. Developing methods: MB, JH, WH TK CB Data analysis: MB, JH SM. Preparation of figures and tables: MB Conducting the research, MB, CB, JH, SM data

interpretation, MB JH, SM, DW, WH, TK writing: MB, SM, JH, WH, TK, DW, CB.

ACKNOWLEDGMENTS

The authors would like to thank Rene Delmas for invaluable help with phytoplankton identification and enumeration, Tony Fowler for logistical help with mesocosms, and Pat McSweeney for his help sampling. Open access publishing facilitated by University of Technology Sydney, as part of the Wiley - University of Technology Sydney agreement via the Council of Australian University Librarians.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). *Permanova+ for Primer: Guide to software and statistical methods*. PRIMER-E.

APHA. (2005). *Standard methods for the examination of water and wastewater* (21st ed.). American Public Health Association.

Baldwin, D. S. (1999). Dissolved organic matter and phosphorus leached from fresh and 'terrestrially' aged river red gum leaves: Implications for assessing river-floodplain interactions. *Freshwater Biology*, *41*, 675–685. https://doi.org/10.1046/j.1365-2427.1999.00404.x

Baldwin, D. S., Colloff, M. J., Mitrovic, S. M., Bond, N. R., & Wolfenden, B. (2016). Restoring dissolved organic carbon subsidies from floodplains to lowland river food webs: A role for environmental flows? *Marine and Freshwater Research*, *67*(9), 1387–1399. https://doi.org/10.1071/MF15382

Berggren, M., Laudon, H., Haei, M., Strom, L., & Jansson, M. (2010). Efficient aquatic bacterial metabolism of dissolved low-molecular-weight compounds from terrestrial sources. *ISME Journal*, *4*, 408–416.

Berggren, M., Ström, L., Laudon, H., Karlsson, J., Jonsson, A., Giesler, R., Bergström, A. K., & Jansson, M. (2010). Lake secondary production fueled by rapid transfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. *Ecology Letters*, *13*(7), 870–880. https://doi.org/10.1111/j.1461-0248.2010.01483.x

Berggren, M., Ziegler, S. E., St-Gelais, N. F., Beisner, B. E., & Del Giorgio, P. A. (2014). Erratum: Contrasting patterns of allochthony among three major groups of crustacean zooplankton in boreal and temperate lakes (Ecology (2014) 95 (1947–1959)). *Ecology*, *95*(11), 3230.

Blumenshine, S. C., & Hambright, K. D. (2003). Top-down control in pelagic systems: A role for invertebrate predation. *Hydrobiologia*, *491*, 347–356. https://doi.org/10.1023/A:1024422121048

Brett, M. T., Arhonditsis, G. B., Chandra, S., & Kainz, M. J. (2012). Mass flux calculations show strong Allochthonous support of freshwater zooplankton production is unlikely. *PLoS One*, *7*(6), 1–10. https://doi.org/10.1371/journal.pone.0039508

Brett, M. T., Kainz, M. J., Taipale, S. J., & Seshan, H. (2009). Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences*, *106*(50), 21197–21201. https://doi.org/10.1073/pnas.0904129106

Bunn, S. E., Davies, P. M., & Winning, M. (2003). Sources of organic carbon supporting the food web of an arid zone floodplain river. *Freshwater Biology*, *48*, 619–635.

Burford, M. A., Cook, A. J., Fellows, C. S., Balcombe, S. R., & Bunn, S. E. (2008). Sources of carbon fuelling production in an arid floodplain river. *Marine and Freshwater Research*, *59*(3), 224–234. https://doi.org/10.1071/MF07159

Carney, R. L., Seymour, J. R., Westhorpe, D., & Mitrovic, S. M. (2016). Lotic bacterioplankton and phytoplankton community changes under dissolved organic-carbon amendment: Evidence for competition for nutrients. *Marine and Freshwater Research*, *67*(9), 1362–1373. https://doi.org/10.1071/MF15372

Cole, J. J., Carpenter, S. R., Kitchell, J., Pace, M. L., Solomon, C. T., & Weidel, B. (2011). Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(5), 1975–1980. https://doi.org/10.1073/pnas.1012807108

Danger, M., Oumarou, C., Benest, D., & Lacroix, G. (2007). Bacteria can control stoichiometry and nutrient limitation of phytoplankton. *Functional Ecology*, *21*, 202–210. https://doi.org/10.1111/j.1365-2435.2006.01222.x

Degerman, R., Lefébure, R., Byström, P., Båmstedt, U., Larsson, S., & Andersson, A. (2018). Food web interactions determine energy transfer efficiency and top consumer responses to inputs of dissolved organic carbon. *Hydrobiologia*, *805*(1), 131–146. https://doi.org/10.1007/s10750-017-3298-9

Drakare, S., Blomqvist, P., Bergström, A. K., & Jansson, M. (2002). Primary production and phytoplankton composition in relation to DOC input and bacterioplankton production in humic Lake Örträsket. *Freshwater Biology*, *47*(1), 41–52. https://doi.org/10.1046/j.1365-2427.2002.00779.x

Entwisle, T. J., Sonneman, J. A., & Lewis, S. H. (1997). Freshwater algae in Australia, Sainty and Associates, Monash University, Australia.

Faithfull, C., Huss, M., Vrede, T., Karlsson, J., & Bergström, A. K. (2012). Transfer of bacterial production based on labile carbon to higher trophic levels in an oligotrophic pelagic system. *Canadian Journal of Fisheries and Aquatic Sciences*, *69*(1), 85–93. https://doi.org/10.1139/F2011-142

Faithfull, C. L., Huss, M., Vrede, T., & Bergström, A. K. (2011). Bottom-up carbon subsidies and top-down predation pressure interact to affect aquatic food web structure. *Oikos*, *120*(2), 311–320. https://doi.org/10.1111/j.1600-0706.2010.18683.x

Flynn, K. J., Stoecker, D. K., Mitra, A., Raven, J. A., Glibert, P. M., Hansen, P. J., Granéli, E., & Burkholder, J. M. (2013). Misuse of the phytoplankton-zooplankton dichotomy: The need to assign organisms as mixotrophs within plankton functional types. *Journal of Plankton Research*, *35*(1), 3–11. https://doi.org/10.1093/plankt/fbs062

Franke, D., Bonnell, E. J., & Ziegler, S. E. (2013). Mineralisation of dissolved organic matter by heterotrophic stream biofilm communities in a large boreal catchment. *Freshwater Biology*, *58*, 2007–2026. https://doi.org/10.1111/fwb.12187

Gawne, B., Merrick, C., Williams, D. G., Rees, G., Oliver, R., Bowen, P. M., Treadwell, S., Beattie, G., Ellis, I., Frankenberg, J., & Lorenz, Z. (2007). Patterns of primary and heterotrophic productivity in an arid lowland river. *River Research and Applications*, *23*, 1070–1087. https://doi.org/10.1002/rra.1033

Hansson, T. H., Grossart, H. P., del Giorgio, P. A., St-Gelais, N. F., & Beisner, B. E. (2019). Environmental drivers of mixotrophs in boreal lakes. *Limnology and Oceanography*, *64*(4), 1688–1705. https://doi.org/10.1002/lno.11144

Hiltunen, M., Honkanen, M., Taipale, S., Strandberg, U., & Kankaala, P. (2017). Trophic upgrading via the microbial food web may link terrestrial dissolved organic matter to daphnia. *Journal of Plankton Research*, *39*(6), 861–869. https://doi.org/10.1093/plankt/fbx050

Hitchcock, J. N., & Mitrovic, S. M. (2013). Different resource limitation by carbon, nitrogen and phosphorus between base flow and high flow conditions for estuarine bacteria and phytoplankton. *Estuarine, Coastal and Shelf Science*, *135*, 106–115. https://doi.org/10.1016/j.ecss.2013.05.001

Hitchcock, J. N., & Mitrovic, S. M. (2015). After the flood: Changing dissolved organic carbon bioavailability and bacterial growth following inflows to estuaries. *Biogeochemistry*, *124*(1), 219–233. https://doi.org/10.1007/s10533-015-0094-3

Hitchcock, J. N., Mitrovic, S. M., Hadwen, W. L., Roelke, D. L., Growns, I. O., & Rohlfs, A. M. (2016). Terrestrial dissolved organic carbon subsidizes estuarine zooplankton: An in situ mesocosm study. *Limnology and Oceanography*, *61*(1), 254–267. https://doi.org/10.1002/lno.10207

Jäger, C. G., Vrede, T., Persson, L., & Jansson, M. (2014). Interactions between metazoans, autotrophs, mixotrophs and bacterioplankton in nutrient-depleted high DOC environments: A long-term experiment. *Freshwater Biology*, *59*(8), 1596–1607. https://doi.org/10.1111/fwb.12366

Jansson, M., Bergstrom, A. K., Blomqvist, P., & Drakare, S. (2000). Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology*, *81*, 3250–3255. https://doi.org/10.1890/0012-9658(2000)081[3250:AOCAPB]2.0.CO;2

Jansson, M., Persson, L., De Roos, A. M., Jones, R. I., & Tranvik, L. J. (2007). Terrestrial carbon and intraspecific size-variation shape lake ecosystems. *Trends in Ecology and Evolution*, *22*, 316–322. https://doi.org/10.1016/j.tree.2007.02.015

Jones, R. I., Kankaala, P., Nykänen, H., Peura, S., Rask, M., & Vesala, S. (2018). Whole-Lake sugar addition demonstrates trophic transfer of dissolved organic carbon to top consumers. *Ecosystems*, *21*(3), 495–506. https://doi.org/10.1007/s10021-017-0164-6

Junk, W. J., Bayley, P. B., & Sparks, R. (1989). Junk et al. 1989 – the flood pulse concept in river-floodplain systems.Pdf. *Canadian Journal of Fisheries and Aquatic Science*, *106*, 110–127.

Jürgens, K., & Jeppesen, E. (2000). The impact of metazooplankton on the structure of the microbial food web in a shallow, hypertrophic lake. *Journal of Plankton Research*, *22*, 1047–1070. https://doi.org/10.1093/plankt/22.6.1047

Kamjunke, N., Henrichs, T., & Gaedke, U. (2007). Phosphorus gain by bacterivory promotes the mixotrophic flagellate *Dinobryon* spp. during re-oligotrophication. *Journal of Plankton Research*, *29*, 39–46. https://doi.org/10.1093/plankt/fbl054

Karlsson, J., Bystrom, P., Ask, J., Ask, P., Persson, L., & Jansson, M. (2009). Light limitation of nutrient-poor lake ecosystems. *Nature*, *460*, 506–509.

Karlsson, J., Jonsson, A., Meili, M., & Jansson, M. (2003). Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. *Limnology and Oceanography*, *48*, 269–276. https://doi.org/10.4319/lo.2003.48.1.0269

Karlsson, J., Lymer, D., Vrede, K., & Jansson, M. (2007). Differences in efficiency of carbon transfer from dissolved organic carbon to two zooplankton groups: An enclosure experiment in an oligotrophic lake. *Aquatic Sciences*, *69*(1), 108–114. https://doi.org/10.1007/s00027-007-0913-2

Katechakis, A., Haseneder, T., Kling, R., & Stibor, H. (2005). Mixotrophic versus photoautotrophic specialist algae as food for zooplankton: The light: Nutrient hypothesis might not hold for mixotrophs. *Limnology and Oceanography*, *50*(4), 1290–1299. https://doi.org/10.4319/lo.2005.50.4.1290

Kirk, J. T. O. (1976). Yellow substance (Gelbstoff) and its contribution to the attenuation of photosynthetically active radiation in some inland and coastal south-eastern Australian waters. *Aust J Mar Freshw Res*, *27*, 61–71.

Kobayashi, T., Shiel, R. J., Gibbs, P., & Dixon, P. I. (1998). Freshwater zooplankton in the Hawkesbury-Nepean River: Comparison of community structure with other rivers. *Hydrobiologia*, *377*, 133–145. https://doi.org/10.1023/a:1003240511366

Lefebure, R., Degerman, R., Andersson, A., Larsson, S., Eriksson, L. O., Bamstedt, U., & Bystrom, P. (2013). Impacts of elevated terrestrial nutrient loads and temperature on pelagic food-web efficiency and fish production. *Global Change Biology*, *19*, 1358–1372.

Marcarelli, A. M., Baxter, C. V., Mineau, M. M., & Hall, R. O. (2011). Quantity and quality: Unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. *Ecology*, *92*, 1215–1225. https://doi.org/10.1890/10-2240.1

McMeans, B. C., Koussoroplis, A. M., Arts, M., & Kainz, M. J. (2015). Terrestrial dissolved organic matter supports growth and reproduction of *Daphnia magna* when algae are limiting. *Journal of Plankton Research*, *37*(6), 1201–1209. https://doi.org/10.1093/plankt/fbv083

Mitrovic, S. M., Westhorpe, D. P., Kobayashi, T., Baldwin, D. S., Ryan, D., & Hitchcock, J. N. (2014). Short-term changes in zooplankton density and community structure in response to different sources of dissolved organic carbon in an unconstrained lowland river: Evidence for food web support. *Journal of Plankton Research*, *36*(6), 1488–1500. https://doi.org/10.1093/plankt/fbu072

Nielsen, D. L., Cook, R. A., Ning, N., Gawne, B., & Petrie, R. (2016). Carbon and nutrient subsidies to a lowland river following floodplain inundation. *Marine and Freshwater Research*, *67*(9), 1302–1312. https://doi.org/10.1071/MF14390

O'Connell, M., Baldwin, D. S., Robertson, A. I., & Rees, G. (2000). Release and bioavailability of dissolved organic matter from floodplain litter: Influence of origin and oxygen levels. *Freshwater Biology*, *45*(3), 333–342.

Pace, M. L., Cole, J. J., Carpenter, S. R., Kitchell, J. F., Hodgson, J. R., Van De Bogert, M. C., Bade, D. L., Kritzberg, E. S., & Bastviken, D. (2004). Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature*, *427*(6971), 240–243. https://doi.org/10.1038/nature02227

Patterson, D. J. (1996). *Free-living freshwater protozoa: A colour guide*. Manson publishing.

Prescott, G. G. (1978). *How to know the freshwater algae* (3rd ed.). WCB/Mcgraw-Hill.

Robertson, A. I., Bunn, S. E., Boon, P. I., & Walker, K. F. (1999). Sources, sinks and transformations of organic carbon in Australian floodplain rivers. *Marine and Freshwater Research*, *50*(8), 813–829.

Shabarova, T., Salcher, M. M., Porcal, P., Znachor, P., Nedoma, J., Grossart, H. P., Seďa, J., Hejzlar, J., & Šimek, K. (2021). Recovery of freshwater microbial communities after extreme rain events is mediated by cyclic succession. *Nature Microbiology*, *6*, 479–488. https://doi.org/10.1038/s41564-020-00852-1

Shiel, R. J. (1995). A guide to the identification of rotifers, cladocerans, and copepods from Australian inland waters. Cooperative research centre for freshwater ecology, Australia.

Sommer, U., & Sommer, F. (2006). Cladocerans versus copepods: The cause of contrasting top-down controls on freshwater and marine phytoplankton. *Oecologia*, *147*, 183–194. https://doi.org/10.1007/s00442-005-0320-0

Sommer, U., Stibor, H., Katechakis, A., Sommer, F., & Hansen, T. (2002). Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production: Primary production. *Hydrobiologia*, *484*, 11–20. https://doi.org/10.1023/A:1021340601986

Tanentzap, A. J., Kielstra, B. W., Wilkinson, G. M., Berggren, M., Craig, N., Del Giorgio, P. A., Grey, J., Gunn, J. M., Jones, S. E., Karlsson, J., Solomon, C. T., & Pace, M. L. (2017). Terrestrial support of lake food webs: Synthesis reveals controls over cross-ecosystem resource use. *Science Advances*, *3*, e1601765. https://doi.org/10.1126/sciadv.1601765

Thorp, J. H., & Delong, M. D. (1994). The riverine productivity model: An heuristic view of carbon sources and organic processing in large river ecosystems. *Oikos*, *70*, 305. https://doi.org/10.2307/3545642

Thorp, J. H., & Delong, M. D. (2002). Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. *Oikos*, *96*, 543–550. https://doi.org/10.1034/j.1600-0706.2002.960315.x

Thrane, J. E., Hessen, D. O., & Andersen, T. (2014). The absorption of light in lakes: Negative impact of dissolved organic carbon on primary productivity. *Ecosystems*, *17*, 1040–1052.

Trochine, C., Villanueva, V. D., Navarro, M. B., Balseiroand, E., & Modenutti, B. (2015). The abundance of mixotrophic algae drives the carbon isotope composition of the copepod Boeckella gracilipes in shallow Patagonian lakes. *Journal of Plankton Research*, *37*(2), 441–451. https://doi.org/10.1093/plankt/fbv005

Ward, B. A., & Follows, M. J. (2016). Marine mixotrophy increases trophic transfer efficiency, mean organism size, and vertical carbon flux. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(11), 2958–2963. https://doi.org/10.1073/pnas.1517118113

Wenzel, A., Bergström, A. K., Jansson, M., & Vrede, T. (2012). Survival, growth and reproduction of Daphnia galeata feeding on single and mixed pseudomonas and Rhodomonas diets. *Freshwater Biology*, *57*(4),835–846.https://doi.org/10.1111/j.1365-2427.2012.02751.x

Westhorpe, D. P., & Mitrovic, S. M. (2012). Dissolved organic carbon mobilisation in relation to variable discharges and environmental flows in a highly regulated lowland river. *Marine and Freshwater Research*, *63*(12), 1218–1230. https://doi.org/10.1071/MF12122

Wetzel, R. G. (1995). Death, detritus, and energy flow in aquatic ecosystems. *Freshwater Biology*, *33*, 83–89.

Whitworth, K. L., Baldwin, D. S., & Kerr, J. L. (2012). Drought, floods and water quality: Drivers of a severe hypoxic Blackwater event in a major river system (the southern Murray–Darling basin, Australia). *Journal of Hydrology*, *450*, 190–198.

Wilken, S., Soares, M., Cordero, P., Ratcovich, J., Ekvall, M., Donk, E., & Hansson, L. A. (2017). Primary producers or consumers? Increasing phytoplankton bacterivory along a gradient of lake warming and browning. *Limnology and Oceanography*, *63*, S142–S155. https://doi.org/10.1002/lno.10728

Worden, A. Z., Follows, M. J., Giovannoni, S. J., Wilken, S., Zimmerman, A. E., & Keeling, P. J. (2015). Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of microbes. *Science*, *347*, 1257594. https://doi.org/10.1126/science.1257594

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How to cite this article: Balzer, M. J., Hitchcock, J. N., Hadwen, W. L., Kobayashi, T., Westhorpe, D. P., Boys, C., & Mitrovic, S. M. (2023). Experimental additions of allochthonous dissolved organic matter reveal multiple trophic pathways to stimulate planktonic food webs. *Freshwater Biology*, *68*, 821–836. https://doi.org/10.1111/fwb.14066