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LETTER

Differential support of lake food webs by three types of terrestrial organic carbon

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Abstract

Organic carbon inputs from outside of ecosystem boundaries potentially subsidize recipient food webs. Four whole-lake additions of dissolved inorganic ^{13}C were made to reveal the pathways of subsidies to lakes from terrestrial dissolved organic carbon (t-DOC), terrestrial particulate organic carbon (t-POC) and terrestrial prey items. Terrestrial DOC, the largest input, was a major subsidy of pelagic bacterial respiration, but little of this bacterial C was passed up the food web. Zooplankton received <2% of their C from the t-DOC to bacteria pathway. Terrestrial POC significantly subsidized the production of both zooplankton and benthic invertebrates, and was passed up the food web to *Chaoborus* and fishes. This route supplied 33–73% of carbon flow to zooplankton and 20–50% to fishes in non-fertilized lakes. Terrestrial prey, by far the smallest input, provided some fishes with >20% of their carbon. The results show that impacts of cross-ecosystem subsidies depend on characteristics of the imported material, the route of entry into the food web, the types of consumers present, and the productivity of the recipient system.

Keywords

^{13}C, allochthony, food web, model, subsidy, whole-lake experiments.

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INTRODUCTION

Ecosystems typically receive materials, including organic matter, from outside of their boundaries. This loading of externally produced (allochthonous) organic matter can subsidize ecosystem metabolism and support the production of consumers of the receiving ecosystem (Vanni & Headworth 2004). Although early ecologists recognized the importance of cross-boundary inputs (Summerhayes & Elton 1923), recent work has focused on quantifying both the magnitudes and significant direct and indirect effects of subsidies on food webs (e.g. Power & Rainey 2000; Polis *et al.* 2004) and ecosystem metabolism (below). The largest allochthonous inputs and hence potential subsidies to most communities and ecosystems is detrital organic matter in dissolved and particulate forms (Polis *et al.* 1997). This material may be directly available or may require transformation by micro-organisms prior to supporting animal consumers in recipient systems. In contrast, smaller fluxes of living organisms across boundaries are often directly available to consumers and numerous studies demonstrate their importance in food webs (e.g. Nakano & Murakami 2001; Sabo & Power 2002). However, for entire ecosystems the relative magnitude and utilization of various types of subsidies remains a poorly understood but significant problem. Theory indicates subsidies can stabilize population dynamics, predator–prey interactions and food webs (DeAngelis 1992; Huxel & McCann 1998; Takimoto *et al.* 2002; Loreau & Holt 2004). Subsidies also indicate interdependence and important connections among ecosystems that are significant for management and conservation (e.g. Ehrenfeld & Toth 1997; Power *et al.* 2004; Douglas *et al.* 2005).

The organic matter imported across ecosystem boundaries can also affect the metabolic balance of the receiving system by providing a substrate for microbial respiration in excess of local primary production (Odum 1956; Webster & Meyer 1999). While these connections and subsidies have had a long history of study in stream ecosystems (e.g. Hynes 1972; Cummins *et al.* 1973; Fisher & Likens 1973) there has been far less work in lakes. Nevertheless, lakes are examples of ecosystems that receive large inputs of allochthonous organic matter with inputs often equal to or exceeding internal primary production (Caraco & Cole 2004). Even though only a small fraction of this allochthonous material is respired, the consequence, for many lakes, is that total respiration exceeds gross primary production (GPP) so that net ecosystem production is negative (del Giorgio *et al.* 1999; Cole *et al.* 2000; Hanson *et al.* 2003; Jonsson *et al.*

2003). Thus, ecosystem metabolism in lakes suggests that allochthonous support of food webs could be significant if degradation of terrestrial carbon is also coupled to consumer production (Cole *et al.* 2002). Studies of lake zooplankton using natural abundances of stable isotopes support this view and indicate zooplankton acquire some of their carbon from terrestrial sources (Meili *et al.* 1996; Jones *et al.* 1999; Grey *et al.* 2001; Karlsson *et al.* 2003).

Terrestrial organic matter may be incorporated into aquatic food webs by several mechanisms. Terrestrially derived dissolved organic carbon (t-DOC) enters lakes in flowing water and is degraded by bacteria that are subsequently consumed by predators (Hessen 1998; Tranvik 1998). Terrestrially derived particulate organic carbon (t-POC) enters lakes by both fluvial and aeolian transport. Terrestrial POC may be available to bacteria through decomposition and to other consumers through direct ingestion (Cole *et al.* 1990; Hessen & Nygaard 1992). Terrestrial-prey items (t-prey) include insects, amphibians, and small reptiles and mammals, which either intentionally or accidentally enter lakes. Fish actively consume these items so t-prey constitutes a variable but significant resource at least in some small lakes (e.g. Carlton & Goldman 1984; Hodgson *et al.* 1993; Hodgson & Hansen 2005). Each of these terrestrial subsidies provides partial support to consumers, which also serve as prey to predators.

Despite some understanding of utilization mechanisms and carbon budgets for lakes, the pathways of utilization and relative magnitudes of the major subsidies have not been compared. Here, the carbon subsidy for the food webs of several lakes is estimated accounting for three terrestrial sources: t-DOC, t-POC and t-prey. Inputs and fates of these sources along with autotrophic production were examined in four, whole-lake ^{13}C additions. Prior work has documented that internal primary production provides only partial support to consumer production in small, low-nutrient lakes (Kritzberg *et al.* 2004; Pace *et al.* 2004; Carpenter *et al.* 2005). This paper adds to a growing literature on terrestrial subsidies by specifically analysing the pathways and relative importance of the three ultimate sources of terrestrial subsidies, t-DOC, t-POC and t-prey, in supporting ecosystem respiration and consumer consumption. The analysis demonstrates that the three kinds of terrestrial carbon make important contributions to aquatic consumers through distinctly different pathways.

METHODS

^{13}C additions and study lakes

$NaH^{13}CO_3$ was added daily to upper mixed layer of Paul, Peter and Tuesday lakes during summer for 5–6 weeks (Kritzberg *et al.* 2004, 2005; Pace *et al.* 2004; Carpenter *et al.* 2005). The enrichments elevated the $\delta^{13}C$ of the dissolved inorganic C (DIC) creating a large contrast between the organic matter produced by (or derived from) primary producers within the lake and terrestrially derived organic sources. The lakes were chosen to provide contrasts in DOC concentrations (an indicator of allochthonous inputs), fish assemblages and nutrient enrichment. The latter contrast was created by fertilizing one of the lakes (Peter Lake) with inorganic nitrogen and phosphorus to stimulate primary production and autochthonous food web pathways.

Paul, Peter and Tuesday lakes are located at the University of Notre Dame Environmental Research Center (89°32′ W, 46°13′ N) and described in detail in Carpenter & Kitchell (1993). The lakes are small (0.9–2.5 ha), soft water systems with negligible growth of rooted aquatic vegetation (macrophytes), moderate to high DOC (0.3–0.7 mmol C L^{-1} or 4–8 mg C L^{-1}) and low concentrations of DIC from 80 to 140 μM. All lakes are strongly stratified with shallow thermoclines (3–4 m), anoxic hypolimnia, and primary production dominated by phytoplankton and periphyton.

Peter Lake was enriched twice with ^{13}C in separate years. Peter Lake was fertilized in the second ^{13}C manipulation (hereafter Peter +N&P) with additions of inorganic N (NH_4NO_3) and P (H_3PO_4) at an N : P atomic ratio of 25 (details in Carpenter *et al.* 2005). Primary producers were stimulated prior to ^{13}C addition by adding 0.69 mmol P m^{-2} and 18.9 mmol N m^{-2} in a single dose on 3 June 2002. Daily additions of 0.11 mmol P m^{-2} day^{-1} and 2.7 mmol N m^{-2} day^{-1} were subsequently made from 10 June to 25 August. For the other three manipulations only the $\delta^{13}C$ of DIC was elevated. Loadings were 0.24, 0.35, 0.25 and 0.61 mol ^{13}C day^{-1} to Paul, Peter, Tuesday and Peter +N&P lakes respectively. In all cases the $\delta^{13}C$ increased the total DIC by <1% and had no measurable effect on pH.

Measurement of ^{13}C

^{13}C content of the major carbon pools was measured before, during and after the tracer addition, at either daily (DIC and POC), weekly [zooplankton, *Chaoborus* spp., small fish, benthic algae (periphyton), DOC] or at longer intervals (larger fish and benthic invertebrates). Detailed methods for sampling and preparation of materials for ^{13}C analysis are summarized elsewhere (Cole *et al.* 2002; Kritzberg *et al.* 2004; Pace *et al.* 2004; Carpenter *et al.* 2005). The isotopic values for some key components came from specialized studies. For example, the measured isotopic signals for pelagic bacteria were obtained from *in situ* growth experiments in dialysis cultures during the ^{13}C additions (Kritzberg *et al.* 2004). The isotopic values for phytoplankton were obtained from the $\delta^{13}C$ of the CO_2 moiety of the DIC and estimates of isotopic discrimination during photosynthesis

by phytoplankton (Bade & Cole 2006; Bade *et al.* 2006). ^{13}C was measured using mass spectrometers as previously described and reported here as $\delta^{13}C$ (i.e. ‰ values relative to a standard, see Carpenter *et al.* 2005).

Additional measurements of standing stocks and fluxes

To support the dual isotope flow (DIF) model (described below), the standing stocks of carbon pools and many of the fluxes among these pools were measured. These measurements included: daily concentrations of DIC and pCO_2; weekly concentrations of chlorophyll *a* (an indicator of phytoplankton biomass), DOC, POC, the abundance and species composition of crustacean zooplankton and the phantom midge, *Chaoborus* (several species), bacterial production, and dark-bottle pelagic respiration. The abundance, size structure and growth rates of the dominant fish species were also measured in each lake. DOC is measured as organic C which passes a 0.7-μm pore glass fibre filter; particulate organic C is organic C < 153 μm that is retained by the glass fibre filter. Terrestrial prey (t-prey) items are identified to species and life stage from the stomach contents of fishes. Methods for these measurements are described elsewhere (Carpenter *et al.* 2005) and provided in an on-line manual (http://www.ecostudies.org/cascade). GPP and total system respiration (R_{tot}) were derived from continuous deployment of YSI sondes that recorded oxygen concentration and temperature (along with pH) at 5-min intervals following methods in Cole *et al.* (2000, 2002) and Hanson *et al.* (2003). GPP estimates include the production of both phytoplankton and benthic microalgae integrated to the bottom of the upper mixed layer. R_{tot}, measured this way, includes the respiration of all autotrophs and heterotrophs in both the water column and sediments to the bottom of the mixed layer. Mixed layer depths were estimated from depth profiles of temperature made weekly. The rate of gas exchange was obtained from direct measurements of the gas piston velocity (k_{600}) using a whole-lake SF_6 addition and wind-based estimates from continuous lake-side wind measurements (Wanninkhof *et al.* 1985; Cole & Caraco 1998; Bade & Cole 2006).

The DIF model

The DIF model was developed to simulate the flow of C (^{12}C + ^{13}C) and ^{13}C. The ecosystem was partitioned into 12 compartments: DIC, DOC, pelagic bacteria, phytoplankton, detrital POC, zooplankton, *Chaoborus*, periphyton and three fish compartments specific to the dominants in each lake. Two differential equations, one for C (i.e. ^{12}C + ^{13}C) and one for ^{13}C, describe the mass balance of carbon for each compartment (Appendix S1). Model parameters were derived for each lake either from direct measurements, calculations from measurements, literature estimates or in some cases by fitting to observed ^{13}C time series (Appendix S1). The boundaries of the ecosystem for the purposes of the model were the air–water interface to the base of the mixed layer encompassing all sediments above the mixed layer of the lake.

Three fish compartments were modelled in each lake. In Paul Lake the three compartments were age classes [young of year, juveniles (age 1 +), and adults] of a single dominant species largemouth bass (*Micropterus salmoides*). In Peter Lake the fish compartments were pumpkinseeds (*Lepomis gibbosus*), sticklebacks (*Gasterosteus aculeutas*) and fathead minnows (*Pimephales promelas*). The dominant species of Tuesday Lake were golden shiners (*Notemigonus chrysoleucas*), sticklebacks and fathead minnows. Some terms in the fish carbon balances (e.g. ingestion of prey items, respiration and egestion) were obtained using a bioenergetics model (Hanson *et al.* 1997) augmented in some cases by measured diets (Appendix S1). Growth and biomass dynamics were measured directly and interpolated to daily values input to the bioenergetics model.

Performance of the model was evaluated in several ways. First, fluxes simulated by the model were checked for reasonableness given extensive measurements and knowledge of rates and processes in the lakes. Second, where possible, the DIF model estimates of some parameters (e.g. photosynthetic fractionation) were compared with estimates based on other methods. Third, modelled values of $\delta^{13}C$ for the compartments were compared against measured values using least squares regression of predicted vs. observed $\delta^{13}C$. The values of fitted parameters were arrived at by minimizing the SSE of the predicted vs. observed ^{13}C regressions.

Terrestrial inputs and pathways

The DIF model provides several pathways for the input and utilization of terrestrial organic carbon (Fig. 1). Analysis of these pathways is the central focus of this paper. Terrestrial DOC (t-DOC) is an input to the DOC compartment and hence this compartment contains carbon of both terrestrial and lake origin. This mixture of DOC is consumed by bacteria that are in turn consumed by zooplankton who are the prey of fish and *Chaoborus*. Thus, t-DOC can potentially move through the food web to fish. Terrestrial POC (t-POC) is an input to the detrital POC compartment. This compartment is a potential resource for zooplankton. Detrital POC is also lost by sedimentation where it becomes food for benthos. Thus, t-POC can move through the food web to fish via zooplankton and benthos (Fig. 1). Finally, t-prey is a food resource for the fish compartments (Fig. 1). All fish compartments except young of year largemouth bass (in Paul Lake) consume t-prey as supported by diet

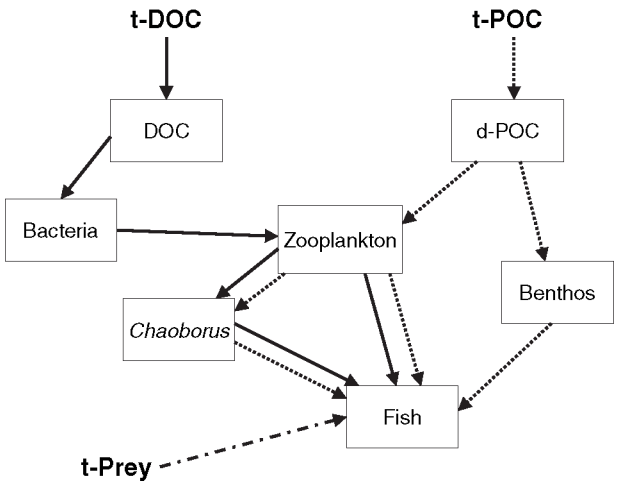

Figure 1 Simplified diagram of pathways for the entry and trophic transfer of terrestrial organic carbon in the dual isotope flow (DIF) model. Each box represents a compartment in the DIF model; the connections of these compartments to the terrestrial inputs are highlighted with arrows. The boxes shown are: DOC (dissolved organic C) which is a mixture of autochthonous and terrestrial sources; dPOC (detrital particulate organic C), also a mixture of autochthonous and terrestrial sources; bacteria (pelagic bacteria); zooplankton (crustacean zooplankton); *Chaoborus*; and benthos (benthic macroinvertebrates). Only the compartments that are connected to terrestrial inputs are shown here. A complete diagram for the DIF model is in Appendix S1, Fig. 1. The three terrestrial C subsidies are: t-DOC (terrestrial DOC); t-POC (terrestrial POC); and, t-prey (terrestrial prey). Each of these terrestrial inputs is connected to the aquatic food web in different ways. The heavy solid arrows show the pathway of entry of t-DOC through the pelagic bacteria. The dashed arrows follow the input and fate of t-POC through zooplankton. The dash-dot arrow follows the consumption of t-prey by fishes. Where more than one kind of arrow is shown (e.g. zooplankton to *Chaoborus*), more than one terrestrial source is utilized. To simplify the diagram pathways of C of autochthonous origin (phytoplankton and benthic algae; see Appendix S1, Fig. 1) are not shown here.

observations (e.g. Hodgson *et al.* 1993; Hodgson & Hansen 2005).

RESULTS

The addition of ^{13}C resulted in a large increase in the ^{13}C content of the DIC in the lakes and, consequently, in both primary producers and consumers (Fig. 2). In all cases primary producers (phytoplankton and benthic algae) became highly labelled but the degree of labelling in the consumer organisms, relative to the primary producers, was quite different among the lakes and type of consumer (Figs 2 and 3). This labelling suggests that an alternative food source such as unlabelled terrestrial carbon is supporting some consumers. The DIF model was used to quantify the use of these alternative sources.

Plots of modelled vs. measured values of ^{13}C for the 12 compartments are shown in Fig. 3. The agreement of modelled with measured values supports the plausibility of the actual fluxes of autochthonous and allochthonous C calculated by the model. The best fits of the DIF model were for the Peter and Paul additions ($R^2 = 0.98$, $n = 136$ and $R^2 = 0.97$, $n = 149$ respectively) and the regressions slopes (+ 1 SE) were close to unity (0.97 + 0.02 and 0.94 + 0.02 respectively). The fit for the Peter +N&P addition was also only slightly lower ($R^2 = 0.92$, $n = 144$, slope = 0.90 + 0.03) an encouraging results considering the rapid dynamics of ^{13}C observed in the lower food web compartments (Fig. 3). While Tuesday had a lower fit the model still explained nearly 80% of the variability in measured values ($R^2 = 0.79$, $n = 151$, slope = 0.96 + 0.04).

Loading of allochthonous and autochthonous organic C

The model provided estimates of organic carbon loading and respiration of the biotic compartments that can be compared with measured values of GPP_{tot} and R_{tot} (Table 1). Model-derived loading of t-DOC and t-POC ranged from 21 to 67 mmol C m^{-2} day^{-1} (Table 1) and differences in loading followed measured differences in concentrations among Paul, Peter and Tuesday lakes. Although t-DOC dominated loading, t-POC accounted for a substantial portion, averaging 19.5% of total terrestrial loading among the lakes (4.6–15.8 mmol C m^{-2} day^{-1}). Total autochthonous primary production (GPP_{tot}) was dominated by phytoplankton and ranged from 34 to 103 mmol C m^{-2} day^{-1} in the lakes. GPP_{tot} was highest in nutrient-enriched Peter Lake where it was fourfold larger than terrestrial loading (Table 1). In the unenriched lakes GPP_{tot} was 1.7 times terrestrial loading in Paul Lake, co-equal to it in Peter Lake (without added nutrients) and only 0.6 times terrestrial loading in Tuesday Lake. The potential importance of a terrestrial subsidy to consumers is larger than the above comparisons suggest because a fraction (13–21% among lakes) of GPP_{tot} is respired by the algae themselves (Table 1).

Respiratory losses of allochthonous and autochthonous C

The three types of terrestrial inputs considered by the DIF model subsidize consumer respiration in different ways (Table 1). Terrestrial DOC was the most important terrestrial subsidy to the respiration of pelagic heterotrophs (pelagic R_h) supporting from 28% (Paul Lake) to 68% (Tuesday Lake) of their respiration. As pelagic bacteria are the only consumers of t-DOC in the model, this result reflects their significance in overall heterotrophic respiration. Overall, t-POC supported less respiration (2–26% of

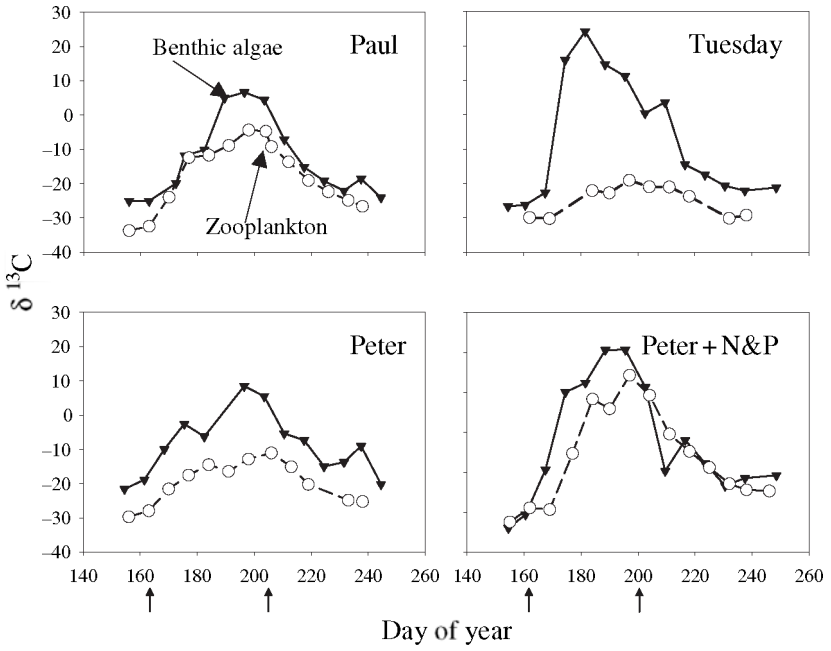

Figure 2 Dynamics of $\delta^{13}C$ for periphyton (solid triangles) and zooplankton (open circles) in the four whole-lake ^{13}C additions. Arrows on *x*-axis indicate the start and end dates of the daily additions of inorganic ^{13}C. For Peter +N&P inorganic N and P were added to simulate primary production (see text).

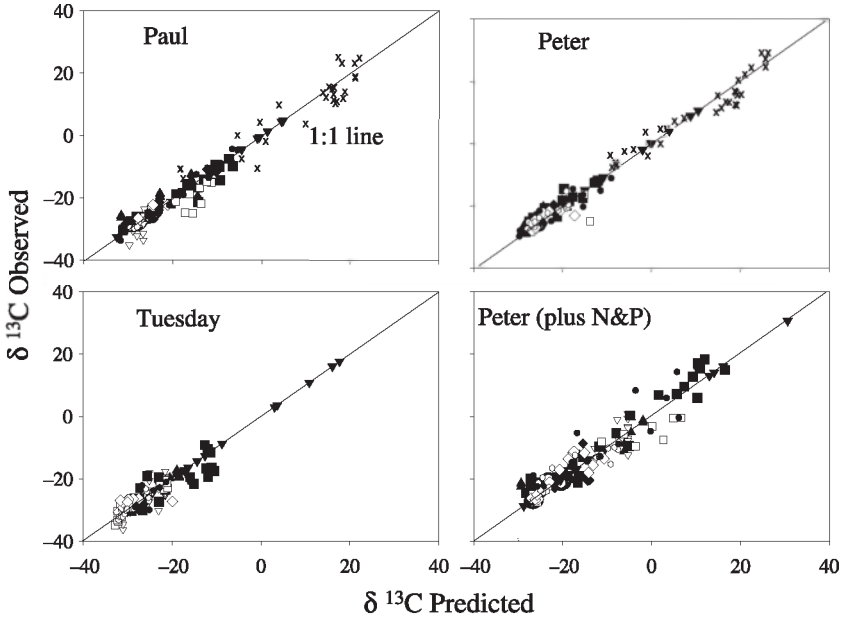

Figure 3 Comparison of the $\delta^{13}C$ predicted by the dual isotope flow (DIF) model with observed values for each lake. In the 12 compartments (Paul and Peter lakes) or 11 compartments (Peter +N&P; Tuesday Lake) are indicated by distinct symbols (below) along with a 1 : 1 line. Least square regressions of predicted vs. observed values were significant at $P < 0.0001$ (see text). ×, DIC; ○, dissolved organic carbon; ▲, pelagic bacteria; ▼, benthic algae; ▽, macroinvertebrates; ■, particulate organic carbon; □, *Chaoborus*; ◆, fish 1; ◇, fish 2; ○, fish 3; ●, zooplankton.

R_h), but this material constituted a respiratory subsidy, especially to zooplankton, which are the major users of t-POC in the water column (below). In Paul Lake t-POC supports nearly as much (5.5 mmol C m^{-2} day^{-1}) pelagic R_h as does t-DOC (6.8 mmol C m^{-2} day^{-1}; Table 1). Terrestrial prey supported only a very small fraction of respiration in all lakes (Table 1), but were highly significant C sources to fishes (below).

While terrestrial inputs were a major source of the carbon ultimately supporting consumer respiration, autochthonous organic carbon from primary production supported a large share of heterotrophic respiration in all cases. In Peter Lake +N&P autochthonous carbon supported 88% of pelagic R_h (38.5 mmol C m^{-2} day^{-1}) or seven times that supported by terrestrial inputs. Even in the absence of nutrients, the autochthonous component of respiration was significant, ranging from 28% of total pelagic R_h in Tuesday Lake to *c.* 60% in Paul and Peter lakes (without nutrient additions).

Epilimnetic sediment respiration (sediment R_h = benthic micro-organisms + meiofauna) comprised 29–47% of total

Table 1 Inputs and respiratory losses of organic C in the four experimental lakes

	Paul	Tuesday	Peter	Peter (+N&P)
Organic C inputs				
GPP_{phyto}	36.3	36.5	28.3	87.1
$GPP_{benthic}$	9.5	0.6	5.6	15.5
GPP_{tot}	45.9	37.2	34.1	102.6
T-DOC	21.9	49.7	31.1	21.1
T-POC	4.7	15.8	6.2	4.7
T-prey	0.20	0.76	0.05	0.17
T-$load_{tot}$	27.7	66.3	38.0	34.6
Respiratory				
R_{auto}	9.7	4.7	6.9	13.3
Pelagic R_h	23.4	16.0	20.2	43.2
Sediment R_h	21.0	15.2	8.32	26.2
R_{tot}	54.1	35.9	35.4	83.1
Pelagic R_h sources				
Autochthonous	10.9	4.4	11.5	38.5
T-DOC	6.8	9.9	5.7	4.1
T-POC	5.5	0.8	2.6	0.4
T-prey	0.20	0.76	0.05	0.17
Sediment R_h sources				
Autochthonous	10.3	4.7	2.1	25.6
Terrestrial	10.7	10.5	6.2	1.0

Values are mmol C m^{-2} day^{-1} and are derived from the DIF model, except for total gross primary production (GPP_{tot}) and total system respiration (R_{tot}) which were directly measured (see text and Appendix S1). The subscripts 'phyto' and 'benthic' for GPP refer to phytoplankton and benthic algae respectively. T-$load_{tot}$ is the total loading of terrestrial carbon from its three sources, terrestrial DOC (t-DOC), terrestrial particles (t-POC) and terrestrial prey items (t-prey). R_{auto} is respiration by autotrophic organisms (sum of phytoplankton and periphyton respiration); R_h, respiration by heterotrophs (consumers). In the bottom two sections the sources of carbon respired by heterotrophs are partitioned. For pelagic heterotrophic R the figure shows C from primary production within the lake (AUTO), and that entering by the three terrestrial routes. The sources for benthic heterotrophic R can be partitioned only into AUTO and terrestrial. The fates of inputs not shown here include outflow and export to the hypolimion. The DIF model also calculates these (see Appendix S1).

heterotrophic R (water column plus sediments; Table 1). Because the model did not explicitly include the dynamics of the microbial and meiofaunal benthos, it is not possible to partition this respiration among the three terrestrial sources, but we can calculate the relative amount supported by total allochthonous and autochthonous sources (Carpenter *et al.* 2005). The DIF model estimated 30–50% of sediment R_h is supported by terrestrial C in the unfertilized lakes. The fertilization of Peter Lake greatly increased the supply of autochthonous C to the benthos and decreased the importance of allochthonous C to <4% (Table 1).

Support of consumers by allochthonous and autochthonous C

The DIF model allows the computation of key flows of terrestrial and autochthonous C sources to consumers including both direct and indirect pathways (Fig. 1; Appendix S1).

Zooplankton and Chaoborus

For all the additions, the flow of t-DOC to bacteria to zooplankton was a very minor pathway, accounting for 1–2% of zooplankton C (Fig. 4a). Carbon of phytoplankton origin was a variable but large source to zooplankton in all cases comprising 25% in Tuesday Lake, *c.* 60% in Peter and Paul lakes and nearly 90% in Peter Lake +N&P (Fig. 4). Terrestrial POC was also an important C source for zooplankton ranging from 73% in Tuesday Lake to near 35% for both Peter and Paul lakes. In nutrient-enriched Peter Lake, t-POC accounted for more than 10% of zooplankton demand (Fig. 4). *Chaoborus*, an important prey for many fish, consumed only zooplankton in the model and the terrestrial subsidy of *Chaoborus* tracks that of zooplankton (data not shown).

Pelagic bacteria

Pelagic bacteria obtain their C entirely from DOC of either terrestrial or in-lake (autochthonous) origin. Terrestrial DOC was a more important C source for bacteria than autochthonously produced DOC in all the additions except Peter +N&P where allochthonous t-DOC supplied 39% of bacterial demand (Fig. 4b). In the other lakes t-DOC accounted for 60–76% of pelagic bacterial demand (Fig. 4b).

Benthic invertebrates

Terrestrial POC was the dominant source of C supporting benthic macroinvertebrates comprising 60–85% of macroinvertebrate production. In the Peter Lake +N&P addition benthic algal production was greatly stimulated (Table 1) and the importance of t-POC was reduced to only 6% of macroinvertebrate production (Fig. 4c).

Fish

In the model, fish had access to t-DOC and t-POC indirectly by preying on the consumers of these sources and their predators (Fig. 1). Fish also consumed t-prey directly. There were large differences in the importance of terrestrial sources and autotrophic C to fishes among lakes and among the different types of fish. In no case was t-DOC an important ultimate C source to any of the fish groups (Fig. 5), reflecting the minor importance of this source to zooplankton. Prey items of terrestrial origin (t-prey) were a significant component for fish in all lakes.

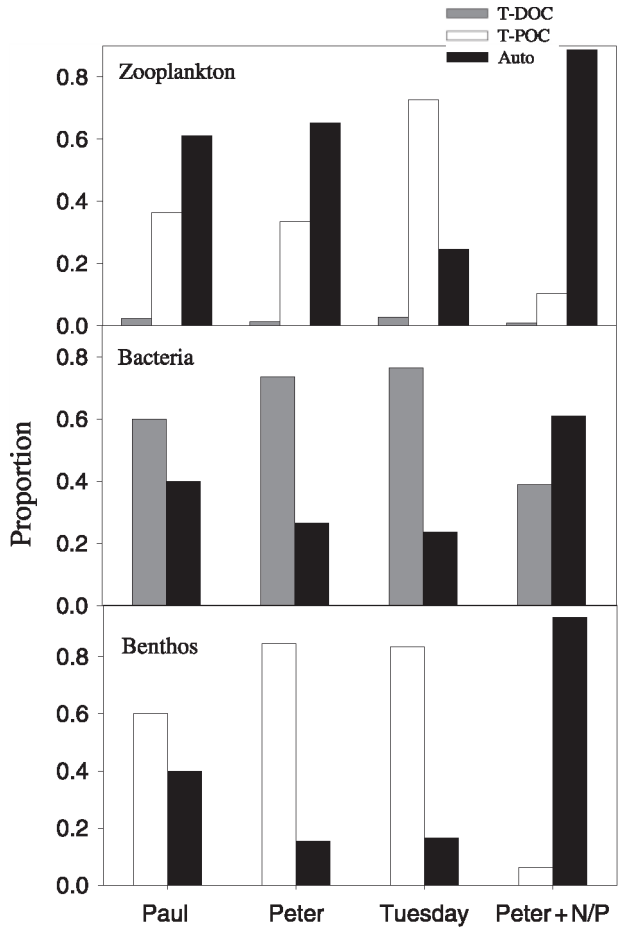

Figure 4 Support of zooplankton, top panel, pelagic bacteria (middle panel) and benthic invertebrates (lower panel) by terrestrial sources (t-dissolved organic C, grey bar; t-particulate organic C, clear bar) and autochthonously produced C (auto, from either phytoplankton or periphyton, solid black bar).

In Paul Lake, where the three fish compartments were age classes of a single species (largemouth bass), autotrophic C was a dominant C source only for young of year (YOY) fish (Fig. 5a). These YOY fish feed primarily on zooplankton before switching to benthic prey as they develop (Post *et al.* 1997). The model indicates that *c.* 40% of YOY C derived from t-POC reflecting the importance of this material to zooplankton, *Chaoborus* and benthos. For juvenile bass which are more benthivorous and piscivorous, t-POC and autotrophic C were co-equal (*c.* 40% each) with the remainder from t-prey (Fig. 5a). In adult fish t-prey, t-POC and autotrophic C are all about co-equal in importance (Fig. 5a). For largemouth bass, then t-POC was always an important subsidy and t-prey became more important as fish increased in size.

Pumpkinseeds and sticklebacks in Peter Lake (without nutrients) used t-POC, t-prey and autotrophic C about equally. Fathead minnows were less dependent on t-prey (Fig. 5b). Fertilization of Peter L (+N&P) greatly increased GPP (Table 1) and the standing stock of phytoplankton (*c.* 10x, see Carpenter *et al.* 2005). The nutrient fertilization resulted in greater use of autochthonous C in most components of the food web (Fig. 4) and decreased importance of the terrestrial subsidies for all three of the fish species (Figs 5b,d). Fertilization did not consistently increase the rate of utilization of terrestrial C by any component of the food web.

In Tuesday Lake t-prey was the dominant source of C supporting golden shiners and sticklebacks accounting for 60–70% of their C consumption (Fig. 5c). For fathead minnows, t-prey were less important than t-POC, as in Peter Lake (above). Overall, fish in Tuesday Lake were only weakly supported by autochthonous C.

DISCUSSION

The DIF model

Our estimates of carbon flows derive from a model that is subject to error because of uncertainty in parameters and in model structure. Nevertheless, there are multiple reasons to have some confidence in the model. First, the model includes a detailed accounting of carbon flows among the major pools of the mixed layers of the lakes. The modelled connections among pools and corresponding estimates of flows are consistent with current understanding of lake carbon cycles (Appendix S1, Figure A1 and Table A3). Second, most of the pools and flows of the model were directly measured, and those not measured were estimated by fitting the model to the measured pools and flows as well as the time series of ^{13}C in the pools (Appendix S1 Table A4A). Thus, the model output is strongly constrained by field observations. Third, there is agreement between independent estimates of parameters that were also fit by the model. For example, Bade *et al.* (2006) estimated phytoplankton fractionation in these lakes by physical separation and obtained results in the same range (12–16‰ in the unenriched lakes and near 0 in Peter Lake +N&P as estimates from the DIF model (f13_4 in Appendix S1 Table A4B). Kritzberg *et al.* (2004, 2005) using *in situ* dialysis culture measured the fraction of algal DOC that supported the growth of pelagic bacteria and obtained very similar results to the DIF model (pX3_auto in Appendix S1 Table A4A). Further, model-fit values for parameters not measured (e.g. zooplankton coefficients of assimilation) were within the range of literature values (Appendix S1 Table A4B). Fourth, the model predictions of ^{13}C dynamics closely match the observations (Fig. 3). Fifth, estimates of allochthony (i.e. the proportion of the carbon flow to a compartment supported by terrestrial carbon)

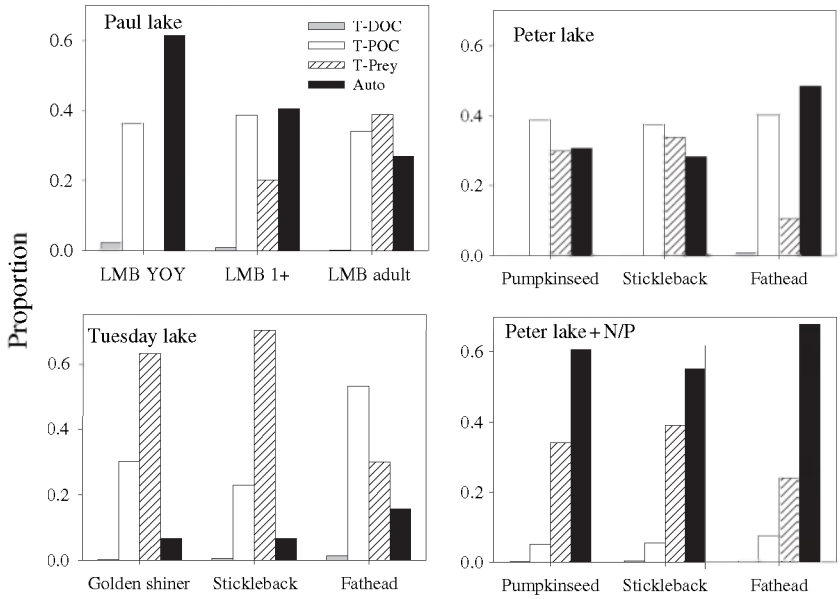

Figure 5 Support of fishes by terrestrial sources. The bars are coded as in Fig. 4 (t-dissolved organic C, grey bar; t-particulate organic C, clear bar; t-prey, hatched bar) and autochthonously produced C (auto, solid black bar). The three groups of fishes modelled in each lake are indicated. LMB, largemouth bass; YOY, young of year; 1 +, juvenile LMB.

from this model were corroborated by two different, independent models fit to the same data (Carpenter *et al.* 2005). Although these other models cannot be used to estimate the specific sources of allochthony presented in this paper, the corroboration of total allochthony lends some confidence to the more detailed accounting of carbon sources presented here. Finally, the estimates of allochthony in the DIF model are robust and not greatly affected by altering the values of key parameters away from those fit by the model. Table A6 and Figure A2 (see Supplementary material) show the effect on model performance of altering values of a few key parameters.

Terrestrial DOC

DOC is the dominant input of terrestrial organic carbon into lakes in general and inputs were similar to GPP in the study lakes except when nutrients were added (Table 1). While pelagic bacteria respire large amounts of t-DOC, they pass very little up the food web. Where allochthony in zooplankton has been reported in other studies, it is often assumed that the pathway is via microbial utilization of t-DOC with subsequent consumption of microbes by zooplankton (Grey *et al.* 2001; Karlsson *et al.* 2003). The present study suggests that zooplankton acquire only a minor terrestrial subsidy by this route. This result is consistent with measured rates of bacterial production and estimated bacterial feeding rates by cladocerans that indicate only a small fraction (4–7%) of zooplankton carbon demand can be supported by bacteria in these lakes. Further, a large fraction (about half) of the DOC acquired by bacteria is of autochthonous rather than allochthonous origin (Kritzberg *et al.* 2004, 2005). Pelagic bacteria, then, pass only a small amount of C up the food web in these lakes and a substantial fraction of that C is not of terrestrial origin.

Terrestrial POC

If the pathway from t-DOC to bacteria to zooplankton is small, how do zooplankton acquire terrestrial C? Both the DIF model and two independent modelling approaches demonstrate that zooplankton in these lakes were heavily subsidized (22–75%) by terrestrial C unless primary production was stimulated by nutrient addition (Cole *et al.* 2002; Carpenter *et al.* 2005). The DIF model suggests that allochthonous support of zooplankton is dominated by direct consumption of terrigenous POC and this subsidy is large in comparison with C of autotrophic origin. Through consumption of zooplankton, their predators (*Chaoborus* and planktivorous fish) also derive a significant subsidy from terrigenous POC. Terrestrial POC was the single largest C source for pumpkinseeds and sticklebacks in Peter Lake (unfertilized). Terrestrial POC was also the largest source of organic carbon to benthic invertebrates, except during experimental enrichment of Peter Lake (Fig. 4). Some unlabelled sediment consumed by benthos may be autochthonous production that occurred prior to the labelling of DIC. However, terrestrial POC is still a major organic carbon source for benthos when sediment carbon is corrected for old autochthonous production (Carpenter *et al.* 2005).

Terrestrial prey

Fish also derive subsidies by directly consuming t-prey items and through piscivory on other fish that consume t-prey. While t-prey is a very minor C input to these lakes, it is a

large subsidy to some fish (Hodgson & Hansen 2005). Only YOY largemouth bass, which are highly planktivorous, were not significantly subsidized by t-prey. In Tuesday Lake t-prey were the dominant C source for both golden shiners and sticklebacks.

Primary production

While most consumers utilize one or more of the three terrestrial subsidies, consumer utilization of primary production from either phytoplankton or benthic algae is important and in many cases larger than all terrestrial sources combined (e.g. zooplankton in Peter and Paul lakes, YOY fish in Paul Lake and fathead minnows in Peter Lake). With a few exceptions (most groups in Tuesday Lake, benthic invertebrates in unenriched Peter Lake) primary production accounted for 30% or more of C demand. The nutrient fertilization of Peter Lake demonstrated that increases in primary production lead to increases in the autotrophic support of secondary production. These results are consistent with the generalization from many comparative studies that consumer biomass and/or productivity increase with primary production (summarized in Kalff 2002). As fertilization had no effect on the rate of processing of allochthonous inputs, this analysis does not support the idea that nutrients or labile co-metabolites from primary production increase the utilization of more recalcitrant terrestrial detritus (Carpenter & Pace 1997; Tranvik 1998). Taken together, the present results in concert with these studies, suggest that terrestrial support of consumers is likely to be most important in low-nutrient, oligotrophic systems. Further, it is possible that the degree of terrestrial subsidy may explain some of the residual variation in regressions between consumer biomass and primary production.

Terrestrial subsidies of ecosystem metabolism

Terrestrial DOC provides substantial support to microbial respiration in the water column in these experiments (e.g. Fig. 4b). As these lakes are often net-heterotrophic (R > GPP), respiration of this terrestrial source would be expected to be significant. While t-POC supports less respiration than does t-DOC, the respiration of t-POC is nevertheless significant and in some cases nearly as large as the respiration of t-DOC (Paul Lake). The respiration of zooplankton is typically co-equal with that of pelagic bacteria (Cole *et al.* 1988) and in these lakes t-POC is an important substrate respired by zooplankton.

General implications

The impacts of subsidies depend on a number of features of the subsidy and the food web including the type of material (e.g. t-DOC vs. t-prey), the flux rate into the recipient system (e.g. large inputs of DOC and small inputs of t-prey), the mode of utilization (e.g. via microbial degradation vs. direct ingestion), the route of entry into the food web, and the temporal variation in the rate of input (e.g. steady loading of t-DOC vs. episodic loading of t-prey). In the present study different consumers were dependent on different forms of terrestrial organic carbon: bacteria on t-DOC; benthos and zooplankton on t-POC; and fishes on t-POC (through consumption of benthos and zooplankton) and t-prey. These subsidies also provided differential support in terms of respiration (primary fate of t-DOC) and production. Hence, assessment of the impact of subsidies on ecosystems requires considering a variety of sources, modes of utilization and food web interactions.

Our study raises several questions concerning how variation in subsidies affects ecosystems. For example, increasing primary production in Peter Lake increased the proportion of autotrophic C supporting consumers. This study does not reveal, however, if increasing terrestrial C inputs to lakes would lead to a similar increase in the terrestrial support of consumers. In addition, while terrestrial organic carbon partially fuels the food webs of the small lakes studied here, would terrestrial carbon be equally important in larger lakes of similar nutrient status? Factors such as watershed size, lake size, loading rates, water residence time and riparian vegetation may be important, and support of food webs might differ considerably among systems.

Our results add to a growing literature on cross-ecosystem subsidies by quantifying the magnitude and pathways by which terrigenous DOC, POC and animal prey subsidize aquatic food webs (Huryn 1996; Nakano & Murakami 2001; Polis *et al.* 2004). The pathways are complex (Fig. 1) and similar complex pathways of subsidization may occur in other ecosystems (Loreau & Holt 2004; Polis *et al.* 2004; McCann *et al.* 2005). It is not known if all pathways have equal impacts on structure, processes and stability of receiving ecosystems. However, the sensitivity of model ecosystems to network structure (Huxel & McCann 1998; Ives *et al.* 2003; Polis *et al.* 2004) suggests that different pathways of subsidization could have important effects on fundamental properties of food web dynamics and carbon cycling. Elucidation of such effects seems to be an expanding frontier of ecological research.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available online for this article from www.Blackwell-Synergy.com:

Appendix S1 Dual isotope flow model.

REFERENCES

Bade, D.L. & Cole, J.J. (2006). Impact of chemically enhanced diffusion on dissolved inorganic carbon isotopes in a fertilized lake. *J. Geophys. Res.*, 111, C01014, doi: 10.1029/2004JC002684.

Bade, D.L., Pace, M.L., Cole, J.J. & Carpenter, S.R. (2006). Can algal photosynthetic fractionation in lakes be predicted from existing models? *Aquat. Sci.* (in press).

Caraco, N.F. & Cole, J.J. (2004). When terrestrial organic matter is sent down the river: importance of allochthonous C inputs to lakes and rivers. In: *Food Webs at the Landscape Level* (eds Polis, G.A., Power, M.E. & Huxel, G.). University of Chicago Press, Chicago, IL, pp. 301–316.

Carlton, R.G. & Goldman, C.R. (1984). Effects of a massive swarm of ants on ammonium concentrations in a subalpine lake. *Hydrobiologia*, 111, 113–117.

Carpenter, S.R. & Kitchell, J.F. (1993). *The Trophic Cascade in Lakes.* Cambridge University Press, Cambridge, MA, 385 pp.

Carpenter, S.R. & Pace, M.L. (1997). Dystrophy and eutrophy in lake ecosystems: implications of fluctuating inputs. *Oikos*, 78, 3–14.

Carpenter, S.R., Cole, J.J., Pace, M.C., Van de Bogert, M., Bade, D.L., Bastviken, D. *et al.* (2005). Ecosystem subsidies: terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. *Ecology*, 86, 2737–2750.

Cole, J.J. & Caraco, N.F. (1998). Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF_6. *Limnol. Oceanogr.*, 43, 647–656.

Cole, J.J., Findlay, S. & Pace, M.L. (1988). Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.*, 43, 1–10.

Cole, J.J., Caraco, N.F. & Likens, G.E. (1990). Short-range atmospheric transport: a significant source of phosphorous to an oligotrophic lake. *Limnol. Oceanogr.*, 35, 1230–1237.

Cole, J.J., Pace, M.L., Carpenter, S.R. & Kitchell, J.F. (2000). Persistence of net heterotrophy in lakes during nutrient addition and food web manipulation. *Limnol. Oceanogr.*, 45, 1718–1730.

Cole, J.J., Carpenter, S.R., Kitchell, J.F. & Pace, M.L. (2002). Pathways of organic C utilization in small lakes: results from a whole-lake ^{13}C addition and coupled model. *Limnol. Oceanogr.*, 47, 1664–1675.

Cummins, K.W., Petersen, R.C., Howard, F.O., Wuycheck, J.C. & Holt, V.I. (1973). The utilization of leaf litter by stream detritivores. *Ecology*, 54, 336–345.

DeAngelis, D.L. (1992). *Dynamics of Nutrient Cycling and Food Webs.* Chapman & Hall, London, UK, 270 pp.

Douglas, M.M., Bunn, S.E. & Davies, P.M. (2005). River and wetland food webs in Australia's wet-dry tropics: general principles and implications for management. *Mar. Freshw. Res.*, 56, 329–342.

Ehrenfeld, J.G. & Toth, L.A. (1997). Restoration ecology and the ecosystem perspective. *Restor. Ecol.*, 5, 307–317.

Fisher, S.G. & Likens, G.E. (1973). Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. *Ecol. Monogr.*, 43, 421–439.

del Giorgio, P.A., Cole, J.J., Caraco, N.F. & Peters, R.H. (1999). Linking planktonic biomass and metabolism to net gas fluxes in northern temperate lakes. *Ecology*, 80, 1422–1431.

Grey, J., Jones, R.I. & Sleep, D. (2001). Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnol. Oceanogr.*, 46, 505–513.

Hanson, P.C., Johnson, D.E., Schindler, D.E. & Kitchell, J.F. (1997). *Fish Bioenergetics 3.0. Sea Grant Technical Report.* University of Wisconsin Sea Grant Institute, Madison, WI.

Hanson, P.C., Bade, D.L., Carpenter, S.R. & Kratz, T.K. (2003). Lake metabolism: relationships with dissolved organic carbon and phosphorus. *Limnol. Oceanogr.*, 48, 1112–1119.

Hessen, D.O. (1998). Food webs and carbon cycling in humic lakes. In: *Aquatic Humic Substances* (eds Hessen, D.O. & Tranvik, L.J.). Springer-Verlag, New York, NY, pp. 285–315.

Hessen, D. & Nygaard, K. (1992). Bacterial transfer of methane and detritus; implications for the pelagic carbon budget and gaseous state. *Arch. Hydrobiol. Beih.*, 37, 139–148.

Hodgson, J.R. & Hansen, E.M. (2005). Terrestrial prey in the diet of largemouth bass, *Micropterus salmoides*, in a small north temperate lake. *J. Freshw. Ecol.*, 20, 793–794.

Hodgson, J.R., He, X. & Kitchell, J.F. (1993). The fish populations. In: *The Tropic Cascade in Lakes* (eds Carpenter, S.R. & Kitchell, J.F.). Cambridge University Press, Cambridge, MA, pp. 43–68.

Huryn, A.D. (1996). An appraisal of the Allen paradox in a New Zealand trout stream. *Limnol. Oceanogr.*, 41, 243–252.

Huxel, G.R. & McCann, K. (1998). Food web stability: the influence of trophic flows across habitats. *Am. Nat.*, 152, 460–469.

Hynes, H.B.N. (1972). *The Ecology of Running Waters.* University of Toronto Press, Toronto, ON, 555 pp.

Ives, A.R., Dennis, B., Cottingham, K.L. & Carpenter, S.R. (2003). Estimating community stability and ecological interactions from time-series data. *Ecol. Monogr.*, 73, 301–330.

Jones, R.I., Grey, J. & Arvola, L. (1999). Stable isotope analysis of zooplankton carbon nutrition in humic lakes. *Oikos*, 86, 97–104.

Jonsson, A., Karlsson, J. & Jansson, M. (2003). Sources of carbon dioxide supersaturation in clearwater and humic lakes in northern Sweden. *Ecosystems*, 6, 224–235.

Kalff, J. (2002). *Limnology: Inland Water Ecosystems.* Prentice Hall, Inc., Upper Saddle River, NJ.

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. *Limnol. Oceanogr.*, 48, 269–276.

Kritzberg, E.S., Cole, J.J., Pace, M.L., Granéli, W. & Bade, D.L. (2004). Autochthonous versus allochthonous carbon sources to bacteria – results from whole lake ^{13}C addition experiments. *Limnol. Oceanogr.*, 49, 588–596.

Kritzberg, E.S., Cole, J.J., Pace, M.L. & Granéli, W. (2005). Does autochthonous primary production drive variability in bacterial metabolism and growth efficiency in lakes dominated by terrestrial C inputs? *Aquat. Microb. Ecol.*, 38, 103–111.

Loreau, M. & Holt, R.D. (2004). Spatial flows and the regulation of ecosystems. *Am. Nat.*, 163, 605–615.

McCann, K.S., Rasmussen, J.B. & Umbanhowar, J. (2005). The dynamics of spatially coupled food webs. *Ecol. Lett.*, 8, 513–523.

Meili, M., Kling, G.W., Fry, B., Bell, R.T. & Ahlgren, I. (1996). Sources and partitioning of organic matter in pelagic microbial food web inferred from the isotopic composition (δ^{13}C and δ^{15}N) of zooplankton species. *Arch. Hydrobiol. Spec. Issues Adv. Limnol.*, 48, 53–61.

Nakano, S. & Murakami, M. (2001). Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. *Proc. Natl Acad. Sci. USA*, 98, 166–179.

Odum, H.T. (1956). Primary production in flowing waters. *Limnol. Oceanogr.*, 1, 102–117.

Pace, M.L., Cole, J.J., Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., Van de Bogert, M.C. *et al.* (2004). Whole lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature*, 427, 240–243.

Polis, G.A., Anderson, W.B. & Holt, R.D. (1997). Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annu. Rev. Ecol. Syst.*, 28, 289–316.

Polis, G.A., Power, M.E. & Huxel, G.R. (eds) (2004). *Food Webs at the Landscape Level.* University of Chicago Press, Chicago, IL.

Post, D.M., Carpenter, S.R. Christensen, D.L., Cottingham, K.L., Kitchell, J.F., Schindler, D.E. *et al.* (1997). Seasonal effects of variable recruitment of a dominant piscivore on pelagic food web structure. *Limnol. Oceanogr.*, 42, 722–729.

Power, M.E. & Rainey, W.E. (2000). Food webs and resource sheds: Towards spatially delimiting trophic interactions. In: *Ecological Consequences of Habitat Heterogeneity* (eds Hutchings, M.J., John, E.A. & Stewart, A.J.A.). Blackwell Scientific, Oxford, UK, pp. 291–314.

Power, M.E., Vanni, M.J., Stapp, P.T. & Polis, G.A. (2004). Subsidy effects on managed ecosystems: implications for sustainable harvest, conservation, and control. In: *Food Webs at the Landscape Level* (eds Polis, G.A., Power, M.E. & Huxel, G.R.). Chicago University Press, Chicago, IL, pp. 387–409.

Sabo, J.L. & Power, M.E. (2002). River-watershed exchange: effects of riverine subsidies on riparian lizards and their terrestrial prey. *Ecology*, 83, 1860–1869.

Summerhayes, V.S. & Elton, C.S. (1923). Contributions to the ecology of Spitsbergen and Bear Island. *J. Ecol.*, 11, 214–287.

Takimoto, G., Iwata, T. & Murakami, M. (2002). Seasonal subsidy stabilizes food web dynamics: balance in a heterogeneous landscape. *Ecol. Res.*, 17, 433–439.

Tranvik, L.J. (1998). Degradation of dissolved organic matter in humic waters by bacteria. In: *Aquatic Humic Substances* (eds Hessen, D.O. & Tranvik, L.J.). Springer-Verlag, New York, NY, pp. 259–283.

Vanni, M.J. & Headworth, J.L. (2004). Cross-habitat transport of nutrients by omnivorous fish along a productivity gradient: integrating watersheds and reservoir food webs. In: *Food Webs at the Landscape Level* (eds Polis, G.A., Power, M.E. & Huxel, G.R.). University of Chicago Press, Chicago, IL, pp. 43–61.

Wanninkhof, R., Ledwell, J.R. & Broecker, W.S. (1985). Gas exchange wind speed relationship measured with sulfur hexflouride on a lake. *Science*, 227, 1224–1226.

Webster, J.R. & Meyer, J.L. (1999). Organic matter budgets for streams: a synthesis. *J. North Am. Benthol. Soc.*, 16, 141–161.

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