Trophic positioning of meiofauna revealed by stable isotopes and food web analyses

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Abstract. Despite important advances in the ecology of river food webs, the strength and nature of the connection between the meio- and macrofaunal components of the web are still debated. Some unresolved issues are the effects of the inclusion of meiofaunal links and their temporal variations on the overall river food web properties, and the significance of autochthonous and allochthonous material for these components. In the present study, we conducted analyses of gut content of macro- and meiofauna and stable isotope analyses of meiofauna to examine seasonal food webs of a chalk stream. The results of the gut content analyses, confirmed by the δ^{13}C signatures, revealed a seasonal shift from a dependence on autochthonous (biofilm) to allochthonous food sources. Here, we demonstrate that aggregating basal or meiofaunal species into single categories affects key web properties such as web size, links, linkage density, and predator–prey ratios. More importantly, seasonal variation in attributes characterized the entire web and these changes persist regardless of taxonomic resolution. Furthermore, our analyses evidenced discrete variations in δ^{15}N across the meiofauna community with a trophic structure that confirms gut content analyses, placing the meiofauna high in the food web. We, therefore, conclude that small-body-sized taxa can occur high in dynamic river food webs, questioning assumptions that trophic position increases with body size and that webs are static.

Key words: benthic algae; feeding plasticity; macrofauna; taxonomic resolution.

Introduction

Ecologists have traditionally used the food web approach to understand the functional complexity of energy flow, stability, and other properties of communities (Thompson et al. 2012). In streams, empirical results based either on stable isotope or gut-content analyses of macrofauna (body size >500 μm) have demonstrated the high levels of autochthony in tropical (Dudgeon et al. 2010) and temperate habitats (Schmid-Araya et al. 2012). These studies contradict the long-held view of heterotrophy and dependency solely on allochthonous detritus proposed by the River Continuum Concept (RCC; Vannote et al. 1980), and agree with aspects of the Riverine Productivity Model (RPM; Thorp and Delong 2002) that established the significance of the autotrophic component in larger rivers (fourth order or higher).

Because of logistic constraints and biases in taxonomic expertise, a trophic species assemblage hardly considered in food web analyses is the freshwater meiofauna (taxa defined in the body-length range ≥50 μm and ≤500 μm). This group exhibits high species diversity and displays

substantial production rates (freshwater systems, 0.8–10.0 g C·m^{-2}·yr^{-1} [Reiss and Schmid-Araya 2010]; marine systems, 0.003–38.8 g C·m^{-2}·yr^{-1} [Burd et al. 2012]). Building upon previous studies that feature the functional role of this group (Schmid-Araya et al. 2002, Stead et al. 2005, Reiss and Schmid-Araya 2011), we examine here their trophic interactions and position in a chalk stream food web.

Stable isotope analysis (SIA) is a widely used technique for determining feeding links of an organism as it reflects and integrates its assimilated diet (Davis et al. 2012). In contrast, gut content analysis (GCA) can be interpreted as a dietary "snapshot" contributing with information on the diversity of the taxonomic and size composition of diets that might be difficult to assess from SIA alone (Davis et al. 2012). Detritivory, omnivory, and diet variability in some consumers complicates the analysis of benthic food webs based on GCA, thus, SIA can enhance the interpretation of food web structure.

Using stable isotopes, marine meiofaunal studies based on species/genus have reported a wide variety of food sources and positioned the group either as primary consumers and/or as predatory (Moens et al. 2005, Vafeiadou et al. 2014). Similarly, the few freshwater studies that applied SIA, although not in combination with CGA, have shown the meiofauna as primary consumers of epilithic biofilms (Majdi et al. 2012, Peters et al. 2012). In freshwaters, the

traditional perception is that species with small body mass occur low in the food web, and, thus, meiofauna would seem to occupy an intermediate position (feeding on basal resources and being fed upon). In contrast, recent marine research has shown that several meiofaunal species are placed at the same trophic level or even higher than their macrofaunal counterparts (Evrard et al. 2010).

On the other hand, food web connectivity displays the trophic interactions within a community based on either assumptions of feeding or GCA. The methodology of defining a link and the level of taxonomic resolution varies widely in the published literature (Thompson and Townsend 2000). As the effort required to highly resolve all taxonomic groups is considered substantial, a great number of these studies have variably resolved trophic links in their webs. To date, there is conflicting evidence if only few food web parameters such as connectance (proportion of links), linkage density (number of links per species), and predator:prey ratios are strongly affected by the inconsistency in the level of resolution used (but see Martinez 1991, Thompson and Townsend 2000). As far as we are aware, no study has yet examined whether changes of food web properties are affected by the inclusion of meiofauna.

In this study, we conducted GCA of macro- and meiofauna, and SIA of meiofauna from a lowland chalk stream in England, UK. Using a highly resolved river food web, we evaluated the effects of resolution (on basal, intermediate, or meiofaunal species) and seasonal variations on food web properties. Furthermore, we assessed seasonal and taxa changes of major meiofaunal energy sources based on $\delta^{13}C$ and meiofaunal trophic position based on $\delta^{15}N$ signatures.

METHODS

Study site

The River Lambourn (51°25′29″ N, 1°21′08″ E) is a circumneutral (pH 7–8) lowland Cretaceous chalk stream draining a rural catchment (234 km^2) of southern England (UK). The Lambourn is fed by a chalk aquifer and, thus, exhibits many characteristics of chalk groundwater-dominated river systems (Pretty et al. 2006). The chalk is a fine-grained, pure (~98% CaCO$_3$), soft, white limestone containing thin marl beds and flints (Weather et al. 2007). The study site was an 80-m stream reach with a mean channel width of 4.5 m, and a maximum water depth of 0.4 m. Mean surface water temperature ranged between 8.4° and 15.4°C, while water conductivity varied between 470 and 540 μS/cm (Tod and Schmid-Araya 2009). The Lambourn is hypernutrified (surface and subsurface 400–500 μmol NO$_3^-$/L, but the streambed is aerobic not serving as a nitrate sink [Pretty et al. 2006]). The riparian vegetation consisted of a wetland and a *Salix* spp. dominated woodland, respectively on the north east and south west riverbank. There were patches of mixed stands of macrophytes, mainly *Ranunculus* spp. and *Berula erecta* (Hudson) Coville.

Sampling for invertebrate densities and gut content analysis

Quantitative sampling of the streambed was carried out with a modified Hess sampler (surface area 0.03 m^2; mesh size 42 μm) between April 2004 and March 2005. Twelve samples were taken at random each month, from within 12 separate 2 × 6 m cross-sectional grids (strata) that covered the whole width of the stream and were spaced evenly across riffle and pool zones. In our analyses, we included not only invertebrates but also all fish caught during sampling. Very low trout densities were recorded in the Lambourn with up to 0.05 individuals/m^2 (Giles et al. 2004) but were absent from our study area. In contrast, bullhead has been recorded with densities up to 8 individuals/m^2 (Mills and Mann 1983).

Samples were kept cool (~5°C) during transport to the lab where they were sieved into size fractions of meiofauna (≥50 μm and ≤500 μm) and macrofauna (>500 μm). Carbonated water was added before fixing as it prevents regurgitation (P. E. Schmid, *unpublished data*). The macroinvertebrates were then immediately preserved in 75% ethanol for later identification and counting, whereas meiofauna fractions were kept cool, and identified and counted within 1–4 d following Stead et al. (2005).

GCA of macro- and meiofaunal species

All invertebrates and fish were enumerated and identified to the lowest possible taxonomic resolution. Feeding interactions were identified by GCA of all invertebrate and vertebrate specimens caught in quantitative samples. Fish and macrofaunal taxa such as Ephemeroptera, Simuliidae, and late instar chironomids were decapitated, dissected, and their gut contents fixed with Euparal microscope slides, while smaller meiofaunal taxa were mounted whole. Slides were then examined under an Olympus BX50 (Olympus Optical, Tokyo, Japan) microscope at 1,000× magnification with oil immersion following Schmid and Schmid-Araya (1997). Whole-mount reference slides and various taxonomic keys were used to identify gut contents. We assessed the gut content of 63% of the species found in the Lambourn because the contribution to relative densities of each of the remaining species was <2% (Tod and Schmid-Araya 2009) and, therefore, too low to carry out meaningful GCA, SIA, and statistical analyses. The total number of individual invertebrates used for gut examination for each season equaled 356 (139 meiofauna) in spring, 562 (236 meiofauna) in summer, 284 (110 meiofauna) in fall, and 182 (66 meiofauna) in winter.

Manipulation of food webs

Four seasonal food web matrices and a summary web were constructed from the data based on GCA. To determine how food web properties were affected by decreasing taxonomic resolution, six further matrices were constructed using the summer web (most species

rich) and the winter web (most species poor). Taxonomic resolution was manipulated by creating three non-replicated levels of resolution by aggregating (1) into categories "algae" and "detritus" (hereafter referred to as the basal web), (2) into categories "insects" and "non-insects" (referred to as the inter web), and (3) meiofaunal species (≤500 μm) as absent (referred to as the meio web).

Web properties based on GCA

For each food web, we assessed the number of meiofaunal species and 16 web properties. Four standard estimates of food web complexity (sensu Martinez 1991, Dunne et al. 2004) were used: (1) species richness [S], (2) number of links between the species [L], (3) links per species [L/S], referred as link density, and (4) connectance C as $C = L/S^2$ or the proportion of all possible links that are realized, referred as directed connectance. Five proportions indicate the types of species in a food web: (1) top (T) species, which lack any predators, (2) intermediate (I) species, (3) basal (B) species, which lack prey items (Martinez 1991), (4) herbivores-detritivores (species feeding on algae, and detritus), and (5) omnivores (species feeding at multiple trophic levels). Five further proportions reveal the type of links between the species: top to basal (T–B), top to intermediate (T–I), intermediate to intermediate (I–I), intermediate to top (I–T), and intermediate to basal (T–B). Finally, we calculated (1) predator:prey ratio, considered cannibalism and any other form of consumption of one organism by another (Dunne et al. 2004), (2) trophic level of all taxa in a web (mean ± SD), and (3) chain length (mean ± SD). The latter was defined as the total number of different chains in a web divided by the sum of all chain lengths (Dunne et al. 2004). A chain is the sequence of links that starts at a basal species and ends with a consumer species. Chain length was also assessed by SIA.

The trophic similarity of seasonal food web matrices was analysed using an agglomerative cluster-analysis (see *Statistical analyses*).

Samples for stable isotope analysis

Twelve additional quantitative Hess samples (six from each of the macrophyte stands and gravel beds) were taken randomly for isotope analysis of meiofauna in each season. Fine particulate organic matter (FPOM) for isotopic source analyses was obtained from the same samples used to collect meiofauna from the streambed. Ceramic tiles (non-glazed) were placed within macrophyte stands at random 1 month prior sampling to allow biofilm colonization (following Bradley et al. 2002). Three replicate samples of biofilm were collected at each sampling occasion. Tiles were scraped with a plastic brush, washed with deionized water, and any invertebrates removed from the resulting slurry. The slurry was then filtered onto pre-combusted and pre-weighed GF/C filters (diameter 25 mm; Whatman), each stored in a separate small airtight plastic bag. Cuttings of the dominant

macrophytes, *Ranunculus* spp. and *Berula erecta*, were taken at each sampling occasion and stored separately in a small plastic bag. All samples of isotopic source material were kept cool during transport to the lab where they were frozen for later SIA preparation.

Meiofauna are capable of extremely high biomass turnover times with up to 1.08 d (i.e., Stead et al. 2005), thus, to ensure better stable isotope signatures of meiofauna, we processed the samples as fast as possible. The reduced column technique for SIA and the preparation procedures are described and discussed in Appendix S1 and Fig. S1.

Statistical analyses

To analyze the trophic similarity of 177 species among seasons, we conducted an agglomerative cluster analysis based on the average group method using as similarity measure the Euclidean distance metric. To test the differences between clusters, we used a SIMPROF approach (Clarke et al. 2008) by testing the similarity of (1) all observed and (2) seasonal combinations of clusters with clusters based on randomly permuted data sets. The test is based on a Pi statistics defined as the absolute deviation of the real similarity profile from the mean of "expected," permuted profiles, which is compared to the null distribution of "simulated," permuted profiles (Clarke et al. 2008). The algorithm for this SIMPROF test was programmed in Delphi 2010 (Embarcadero Technologies, USA). All test results are based on 5000 expected and 4999 simulated random permutations.

A resemblance matrix with key food web variables was created in which between sample distances (factors: seasons and level of taxonomic resolution) were based on Euclidean distance metric calculated on normalized variables. To explore the effects of season and resolution on food web properties, we used multivariate analyses. To detect differences between taxonomic resolutions and between seasons we used permutational ANOVA (PERMANOVA) as this approach is more powerful than other methods to test resemblance matrices (Anderson and Walsh 2013). To test if the variances (distances) of the two seasonal (winter and summer) and two web resolutions (aggregated and non-aggregated) groups are different, the distances of the group members to their respective group median were subject to ANOVA using the PERMDISP approach (Anderson 2006). Following Warton et al. (2012), we also considered if the data displayed differences in location and/or dispersion effects. Location effects indicate a difference on the mean, while dispersion effects indicate a difference in variation of the web properties. Here, we used an approach where model residuals are permuted to generate 4,999 permutation distributions of F values under the null hypothesis of equality of variances between groups. The results of different groups were visualized using non-metric multidimensional scaling (NMDS).

Prior testing isotope signatures for statistically significant differences between seasons and taxa/food-sources, we tested the data for (1) normality using the

Andersen-Darling A^2 test statistics, (2) outliers using the generalized ESD test, and (3) homogeneity of variances using Cochran's C statistic. While taxa such as non-predatory chironomids, harpacticoid copepods, and food sources such as FPOM departed significantly from normality (Appendix S1: Table S1; $P < 0.05$) due to the presence of signature outliers (ESD test; $P < 0.05$), all signature values displayed equality of variances within each tested group (Appendix S1: Table S2; $P > 0.1$). The distribution of non-normal isotope signatures of taxa and food sources fit best to a Gaussian Mixture distribution (A^2 tests; $P > 0.1$). Consequently, we tested seasonal as well as food-source and taxa-specific differences in $\delta^{13}C$ and $\delta^{15}N$ isotope signatures using unbalanced (unequal number of replicated signature measurements), one-way factorial permutation ANOVAs (pmANOVA) considering all possible permutations (exact permutation test) per data set. This permutation test procedure is particularly adequate for (1) normal and non-normal distributed data, (2) low degree of freedom of errors, and (3) data sets containing outliers. We used R language for statistical computing (R Development Core Team 2008) and the R packages vegan 2.2 (Oksanen et al. 2010) and lmPerm (Wheeler 2010).

RESULTS

Food web properties based on GCA

In total, 177 different consumers and food resources were identified in the River Lambourn including 49 different diatom species, 12 other basal resources such as fine particulate organic matter, and leaf material, three types of invertebrate eggs, 112 invertebrate and one fish species (Appendix S1: Table S3). Testing for seasonal structure of trophic similarities among all species demonstrated that the observed similarity profiles of all seasonal clusters (Appendix S1: Fig. S2a–c), and selected cluster combinations significantly departed from random expectations ($P < 0.01$), implying distinct seasonal differences in trophic similarity (Appendix S1: Table S4).

Web size nearly doubled from the winter with 77 species to 133 species in summer, the number of links and linkage density being directly proportional to web size (Table 1). Directed connectance was low and constant across all seasons ranging between 0.04 and 0.05.

Except for the summer season when all proportions of species were equally represented, more basal (range

TABLE 1. Food web properties and percentage of meio- and macrofaunal-sized species estimated for four seasonal webs, a summary web, and six aggregated versions of summer and winter webs with coarsely aggregated basal, intermediate, and meiofauna species from the streambed community of the River Lambourn between 2004 and 2005.

Parameters	SP	SU	FA	WI	Summary	Summer Basal	Summer Inter	Summer Meio	Winter Basal	Winter Inter	Winter Meio
Web properties											
Web size (S)	113	133	89	77	177	86	95	97	44	62	62
No. links (L)	540	824	365	266	1,303	234	455	587	93	187	203
Linkage density (L/S)	4.78	6.20	4.10	3.46	7.36	2.72	4.78	6.05	2.11	3.02	3.27
Directed connectance	0.04	0.05	0.05	0.04	0.04	0.03	0.05	0.06	0.05	0.05	0.05
Chain length											
Mean	2.72	2.89	2.33	2.63	3.25	2.88	3.04	2.87	2.55	2.85	2.63
SD	0.81	0.79	0.47	0.70	0.88	0.81	0.78	0.77	0.70	0.79	0.69
Trophic level											
Mean	1.56	1.66	1.59	1.56	1.64	2.07	1.50	1.53	2.00	1.45	1.45
SD	0.52	0.52	0.52	0.54	0.52	0.30	0.53	0.54	0.35	0.52	0.53
Species proportions											
Basal	0.45	0.37	0.43	0.47	0.40	0.02	0.52	0.51	0.05	0.56	0.57
Intermediate	0.17	0.30	0.18	0.21	0.27	0.47	0.02	0.19	0.39	0.03	0.15
Top consumer	0.38	0.33	0.39	0.33	0.35	0.51	0.46	0.31	0.57	0.40	0.29
Link proportions											
T–B	0.52	0.44	0.63	0.50	0.41	0.36	0.80	0.49	0.46	0.71	0.53
T–I	0.02	0.06	0.07	0.05	0.06	0.20	0.03	0.04	0.15	0.03	0.04
I–I	0.03	0.04	0.00	0.03	0.04	0.15	0.01	0.03	0.10	0.01	0.03
I–B	0.43	0.46	0.30	0.42	0.50	0.30	0.16	0.45	0.29	0.25	0.40
Predator (carnivore)/prey ratio	0.02	0.08	0.05	0.02	0.06	0.10	0.11	0.09	0.09	0.07	0.07
Omnivores	0.04	0.09	0.05	0.07	0.07	0.14	0.12	0.10	0.11	0.08	0.05
Herbivores-detritivores	0.51	0.54	0.53	0.47	0.54	0.84	0.37	0.39	0.84	0.35	0.39
Meiofaunal-sized species (%)	38.7	41.7	39.2	35.7	35.7	41.7	30.4	0.0	35.7	28.0	0.0
Macrofaunal-sized species (%)	61.3	58.3	60.8	64.3	64.3	58.3	69.6	100.0	64.3	72.0	100.0
Meiofauna as top consumers (%)	44.2	31.8	34.3	28.0	24.6	31.8	31.8	0.0	28.0	28.0	0.0

Note: SP, spring; SU, summer; FA, fall; WI, winter; B, basal species; I, intermediate species; M, meiofauna.
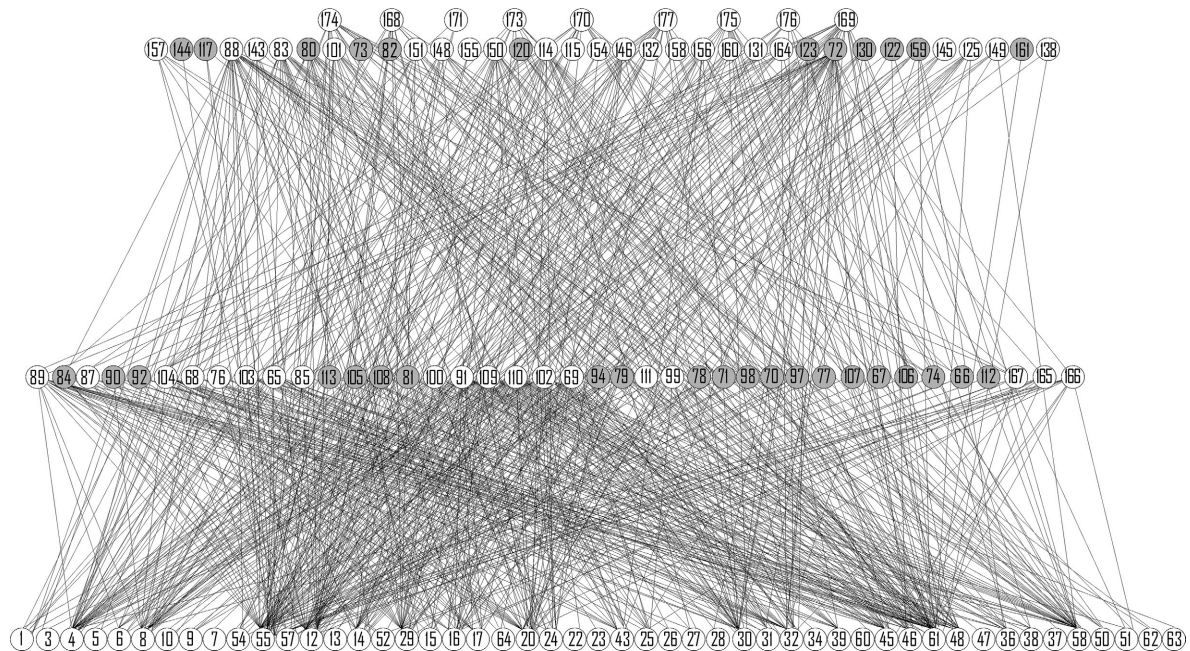
Fig. 1.   Pictorial representation of the food web of the River Lambourn, UK, for summer 2004 (numbers represent food type/species listed in Appendix C). Gray colored bubbles represent meiofaunal-sized taxa.

0.37 and 0.47) and top consumers (range 0.33 and 0.39) inhabited the streambed habitats than intermediate species (Table 1). With the exception of summer when an even distribution of links between top-basal and intermediate-basal species occurred (Table 1, Fig. 1), a notably common feature among all the top consumers was that they fed mainly on basal resources (detritus and diatoms; range 0.50–0.63, Table 1). Furthermore, there was a lack of links between intermediate-to-intermediate species in the fall (Table 1). Linkage density, mean chain length and mean trophic level were highest during the summer coinciding with a higher number of species. Generally, most species were herbivores-detritivores while omnivores (feeding on more than one trophic level) and carnivores were rare in that stream.

In these webs, more than one-third of all invertebrate species belonged to the category meiofauna (range 35.7%–41.7%; Table 1) and they were more abundant than the macrofauna in each season (Appendix S1: Fig. S4). Moreover, the percentage of meiofaunal species within the top consumers was between 28% and 44% (Table 1). The summer web was the species richest (Table 1, Fig. 1) exhibiting a trophic structure with a high number of basal species and with top consumer species (mostly of the herbivore-detritivore category) not being preyed upon by predatory (carnivore) taxa. The web also highlights that meiofaunal-sized species are not only intermediate but also positioned as top consumers (Fig. 1). A low number of trophic levels was reflected in a low mean

chain length (Table 1), and a rather simple web architecture (Fig. 1).

Effects of resolution on the summer and winter web

Poorly resolved basal, intermediate, or meiofaunal food webs showed a reduced web size, as well as changes in the number of links (Table 1). Half the number of links was lost when 42 diatom species were excluded (basal web) for summer and winter seasons (Table 1). In addition, all aggregation protocols produced either an over- or underestimation of linkage density (Table 1). Eliminating basal and meiofaunal species resulted in an overestimation of predator:prey ratios independent of season, although the effect was more pronounced in winter. As these properties (web size, links, linkage density, and predator:prey ratio) displayed more distinct variations among levels of resolution compared to others, we used these key properties for further analyses. PERMANOVA distinguished significant differences in web resolution and seasons (Appendix S1: Table S5), but not in the interaction between the two factors (Appendix S1: Table S5). PERMDISP results highlighted the equality of distances between seasons and between levels of resolution (Appendix S1: Table S6), implying an effect on the mean of the web properties (location) different from a dispersion effect. This is shown in Fig. 2 where season is the predominant effect overlying levels of resolution. Distances between levels of resolution to the observed web are similar among seasons with a distinct separation of the summer basal (SU_{bas}) or winter basal (WI_{bas}) webs from the other two aggregated webs (Fig. 2).
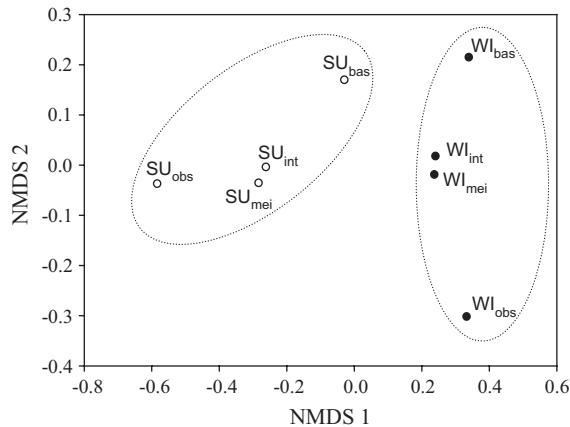
FIG. 2. Nonmetric multidimensional scaling (NMDS) ordination plot based on observed food web properties from the summer (SU_{obs}) and winter (WI_{obs}) seasons and their three aggregated versions (basal [bas], intermediate [int], and meiofauna [mei]) from the streambed community of the River Lambourn between 2004 and 2005.

Meiofauna isotope analysis

Over the four seasons, allochthonous carbon sources with a $\delta^{13}C$ ranging from −32.90‰ to −29.99‰ were on average less depleted than the autochthonous sources, which varied between −38.74‰ and −34.29‰ (Fig. 3). *Ranunculus* spp. showed a depleted $\delta^{13}C$ signature throughout spring to fall while *Berula erecta* maintained a consistently lighter $\delta^{15}N$ signature than biofilm and *Ranunculus* spp. in each season (Fig. 3). However, among the isotopic sources only FPOM displayed significant seasonal changes in $\delta^{13}C$ (pmANOVA $P = 0.003$, Appendix S1: Table S7), but not in $\delta^{15}N$ (pmANOVA $P = 0.708$, Appendix S1: Table S7).

The base line isotopic source data covered the range of meiofauna isotopic signatures in each season well. The mean dry mass per tin cup across all meiofauna samples was 2.26 ± 0.17 µg (mean ± SE) for N, and 5.64 ± 0.44 µg for C, both well above the lower limits required for accuracy in determining isotopic signatures outlined in our method (Appendix S1).

Error bars indicated noticeable variation in $\delta^{13}C$ for each meiofauna group within seasons (Fig. 3), and differences in $\delta^{13}C$ signatures between meiofaunal taxa were marginally significant in the spring season (pmANOVA $P = 0.096$, Appendix S1: Table S8). Seasonally, variation in $\delta^{13}C$ signatures was significant only in harpacticoids (pmANOVA $P = 0.005$, Appendix S1: Table S7) but not for other meiofaunal taxa (pmANOVA $P > 0.1$, Appendix S1: Table S7). Cyclopoid copepods could not be tested as the number of replicates was too small. The seasonal average of $\delta^{13}C$ signatures in Fig. 4 shows ranges for
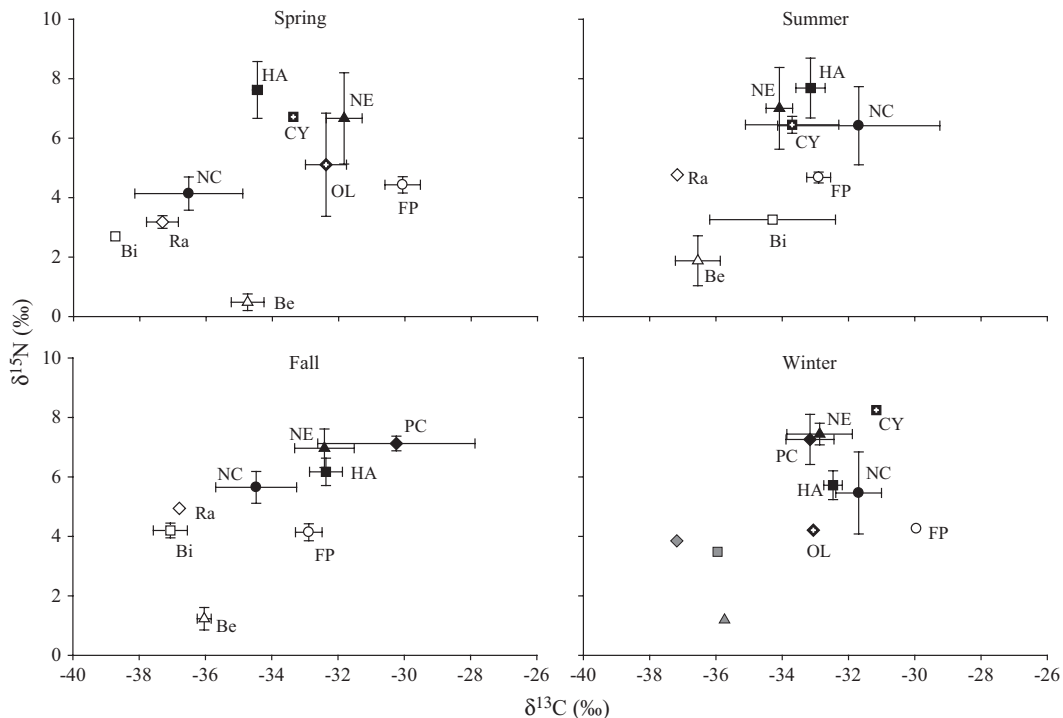

FIG. 3. Variation (mean ± SE) of $\delta^{13}C$ and $\delta^{15}N$ for various meiofaunal groups and their basal resources within the benthos of the River Lambourn during winter 2004 and spring, summer, and fall 2005. Bi, biofilm; Ra, *Ranunculus* spp.; Be, *Berula erecta*; FP, fine particulate organic matter; NC, non-predatory Chironomidae larvae; PC, predatory Chironomidae larvae; HA, harpacticoid copepods; CY, cyclopoida copepods; NE, nematodes; OL, oligochaetes. The gray symbols in the winter plot represent an estimated overall mean across other seasons.
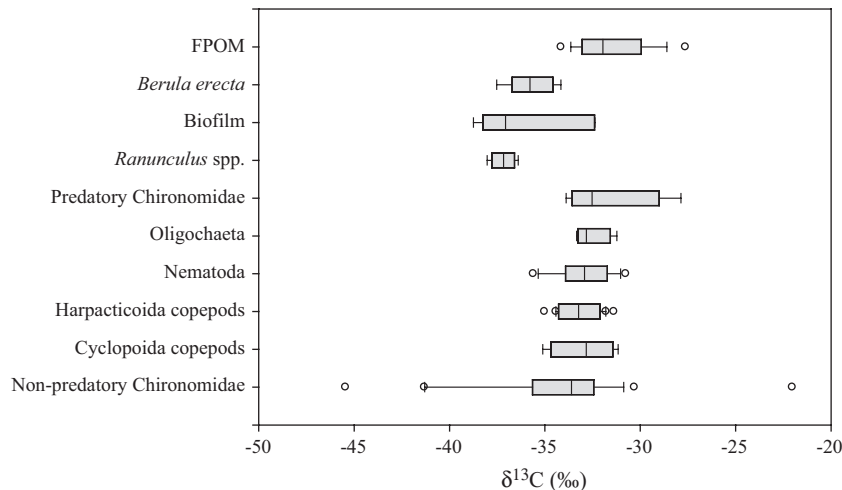
Fig. 4. Box plot of $\delta^{15}C$ signatures of meiofauna and basal resources from the River Lambourn. Midlines are median values, box edges are interquartile ranges, whiskers are minima, and maxima. Open circles mark outliers.

non-predatory chironomids between −36.52‰ and −31.69‰ and those of predatory chironomids between −33.16‰ and −30.24‰.

Overall, the position of most meiofaunal taxa in terms of $\delta^{13}C$ suggests a mixed energy source with a shift from a clear dependence on autochthonous (biofilm) food sources in the summer to an allochthonous food source during the fall (Fig. 3).

Nonsignificant seasonal differences were found in the $\delta^{15}N$ signatures of all meiofaunal taxa (pmANOVA $P > 0.1$, Appendix S1: Table S7). Nitrogen isotope values overlapped among the meiofauna in summer, while in spring, non-predatory chironomids and oligochaetes (primary consumers) were positioned lower than the rest of the meiofauna (Fig. 3). A stepwise increase in $\delta^{15}N$ nitrogen was observed in fall with non-predatory chironomids as primary consumers and predatory chironomids as top consumers (Fig. 3). During winter, oligochaetes were positioned lower than harpacticoids and non-predatory chironomids and these in turn were lower than cyclopoids, nematodes, and predatory chironomids. Applying the standard estimate of 3.4‰ fractionation of $\delta^{15}N$ among trophic levels (Post 2002), we estimated a maximum number of trophic levels between 1.75 and 2.33 in this chalk stream. However, an alternative scenario using the 2.3‰ fractionation of $\delta^{15}N$ of McCutchan et al. (2003) estimated maximum trophic levels between 1.09 and 3.76.

Discussion

This study stresses the importance of temporal variations in food and consumer species composition for a comprehensive understanding of food web structure, asserted by similar changes in trophic structure depicted by CGA and SIA. Although, the binary web had a low number of intermediate species, the meiofauna

contributed with more than one-third of all species to the web. Interestingly, meiofaunal species belonged also to the category of top consumers in this system. The effects of resolution on web properties examined by aggregating either basal, intermediates, and/or meiofaunal species into coarser taxonomic classes resulted in marked effects upon web size, number of links, linkage density, and predator:prey ratios. However, web connectance was insensitive to our aggregation procedure. Seasonal differences in these key web properties together with those for trophic similarity as revealed by PERMANOVA and cluster analysis, respectively, had a pronounced effect on food web structure. PERMANOVA results also highlighted that reductions in taxonomic resolution significantly alter web properties. Key web characteristics are not static properties, having a potentially profound influence on the functional understanding of ecosystems.

CGA results were confirmed by $\delta^{13}C$ signatures suggesting a mixed energy source with a shift from a clear dependence on autochthonous (summer) to allochthonous food sources (fall). The results from the $\delta^{15}N$ signatures reiterated the type of trophic structure, and high positioning of the meiofauna in the food web.

Web properties based on binary approach from GCA

Previous studies have reported food web sizes lower than those found in the current study (see Dunne et al. 2004), and none used such high taxonomic resolution including both basal and meiofaunal species. Highly resolved and complex webs are often dominated in species number by intermediate taxa mostly belonging to the meiofauna (Schmid-Araya et al. 2002, Hillebrand and Shurin 2005), that translates into greater number of links between intermediate species (I–I) in webs of previous studies (Martinez 1991, Schmid-Araya et al. 2002). In contrast, the Lambourn had greater proportions of top

and basal species most of the year while even proportions occurred only in the summer. Another distinction from previous food webs was that more than one-quarter of invertebrate species, categorized as top consumers, were of meiofaunal size, which is a direct consequence of the high resolution level employed.

The number of predatory species was low in the Lambourn but comparable to those of the Duffin Creek (Tavares-Cromar and Williams 1996), implying that some systems are characterized by low predator:prey ratios. Recent findings draw attention to that predator-prey biomass ratios decline when ecosystems are bottom-heavy (Hatton et al. 2015). The assumption that macrofauna and fish can solely describe a food web and its properties may greatly overestimate the true impact of predation. Trout, which is known to feed mostly on drifting terrestrial organisms (Dahl 1998), was absent from our study area, while bullhead, a benthic feeder, consumes on average 2.7 prey items/d (Adamicka 1991), which compares well with our data from the Lambourn with a daily consumption of 3.1 items (J. M. Schmid-Araya, *personal observation*). Comparatively, the likely impact of meiobenthic tanypods is much larger considering that a single individual of a species consumes on average 2.6 ± 0.3 prey items daily (Schmid and Schmid-Araya 1997), which would be equivalent to a mean daily consumption rate of 180.0 prey items/m^2 in the Lambourn. As the top consumer position of meiofauna species demonstrates, it is apparent that their high abundance and species richness reduces the potential impact of the macrofauna and fish predation on the food web in the Lambourn. Finding the meiofauna high in the food web challenges the assumption that small-body-mass taxa occur low in the food web or as an intermediate component and question the simple notion that trophic position increases with body size. In sandy sediments, similar ranges of stable isotopes signatures among macro- and meiofauna indicate that the latter can be placed at the same trophic height as the former (Evrard et al. 2010). If the meiofauna generally plays a major role in energy transfers by being higher up in the food web, will remain elusive as long as food web ecologists do not integrate small-sized organisms in web analyses.

Using the binary approach evidenced a low percentage of omnivores in the Lambourn similar to tropical streams in Hong Kong (Dudgeon et al. 2010) and it might confirm that omnivory is much less common in streams than in lakes (Thompson et al. 2007). Detritivory and omnivory are feeding modes that are difficult to quantify with CGA (see *Caveats*), resulting in an inadequate representation of their importance in food webs (Jepsen and Winemiller 2002).

Taxonomic resolution

In the Lambourn, several key food web properties were sensitive to changes in taxonomic resolution whether the manipulation reduced the number of basal, intermediate,

or meiofaunal species. A decrease in resolution (in whatever form) creates an evident reduction of the number of species involved in a food web (Thompson and Townsend 2000), with a concomitant decrease in web size, number of links, and links per species. Our results agree with previous findings (Martinez 1991, Thompson and Townsend 2000) that a reduction of resolution affected properties such as linkage density and predator:prey ratios. However, contrasting results were found for connectance, where similar to our results, Martinez (1991) depicted it as a robust parameter while Thompson and Townsend (2000) found the opposite.

An important problem in describing food webs is deciding how many and which groups of organisms should be included for a web to represent a meaningful trophic structure (Martinez 1991). Our results are the first to consider the effects of resolution of basal, and particularly intermediate and meiofaunal, species and, thus, await results in other systems. We demonstrate that aggregating basal, intermediate, and meiofaunal species constitute a challenge in the standardization when documenting a web. To skip basal and meiofauna species leads to a crude underestimation of proportions of species, types of links and, thus, bias the interpretation of food web patterns and system's complexity, considering that their contribution to species richness and biomass production is substantial (i.e., Schmid-Araya et al. 2002). However, few studies have examined this aspect. More compelling is that our results are in contrast to previous taxonomic resolution analyses, as we detected significant seasonal variations in key web characteristics emphasizing the dynamics of food webs and, therefore, temporal dynamics ought to be considered prior to generalizing any food web patterns (see McMeans et al. 2015).

Staple isotopes analysis

The general SIA results showed that meiofauna did not exclusively derive carbon from either allochthonous or autochthonous food sources. Grazing of bacteria, algae, detritus, diatoms, and protozoa by lotic meiofauna (Finlay et al. 1993, Hall and Meyer 1998), as well as preying on other meiofauna (Schmid and Schmid-Araya 1997) is well documented. The $\delta^{13}C$ signatures of meiofaunal taxa and food sources fall well within the ranges reported by Trimmer et al. (2009) for the macrofauna (i.e., *Gammarus pulex*, *Simulium*) and their putative resources in the same river. Non-predatory chironomids and harpacticoids showed somewhat depleted $\delta^{13}C$ signatures towards the spring, but were only significant for the latter group. Both seemed reliant on biofilm and *Ranunculus* spp., but their $\delta^{13}C$ signature values were not as low as the ones reported by Trimmer et al. (2009) for cased caddisflies feeding on methane-oxidizing bacteria. We expected that the meiofauna (harpacticoids, oligochaetes, and non-predatory chironomids) could ingest this type of bacteria because of their detritivorous mode of feeding. They inevitably ingest parts of the microbial

biofilm that coats diverse detrital surfaces. At a finer scale of genus/species level, major shifts in the $\delta^{13}C$ content indicated seasonal shifts in the food sources (from algae to *Spartina* detritus) of coastal meiofaunal consumers (Carman and Fry 2002, Moens et al. 2002). Moreover, meiobenthos' resources were different in an estuarine food web in and adjacent to seagrass *Zostera noltii* (Vafeiadou et al. 2014). Thus, meiofaunal trophic plasticity is well known, and in freshwaters, many protozoa (here testate amoebae) and meiofaunal species feed opportunistically and randomly on a wide range of abundant food items (Finlay et al. 1993, Schmid and Schmid-Araya 1997).

The hypernutrified condition of the Lambourn (Pretty et al. 2006) reflected on the $\delta^{15}N$ signatures of the basal resources as these fell well within the ranges from rivers draining agricultural land reported by Peipoch et al. (2012). In the Lambourn, the variation in $\delta^{15}N$ between 4‰ and 8‰ across the meiofauna community was nearly identical to variations (4–9‰) observed for its macroinvertebrates (J. L. Pretty, *personal communication*). These $\delta^{15}N$ signatures are also consistent with values for marsh meiofauna (Carman and Fry 2002).

Despite our coarse taxa approach using SIA, there was a clear difference in $\delta^{15}N$ among the meiofauna with oligochaetes and non-predatory chironomids as primary consumers while nematodes and predatory chironomids were higher in the food chain. Freshwater nematodes feed on a wide range of food items (i.e., bacteria, algae, ciliates, rotifers, nematodes, Naididae oligochaetes; J. M. Schmid-Araya, *personal communication*) similar to their marine counterparts (Moens et al. 2005). Their high trophic position, based on $\delta^{15}N$ ranged seasonally on average between 6.7‰ and 7.4‰, suggests that most nematodes in the Lambourn were omnivores/predators. Strikingly the range of $\delta^{15}N$ values of predatory chironomids of the genus *Conchapelopia* was also narrow, (7.1–7.3‰ seasonal averages) indicating one high trophic level (predator/omnivore). We were able to discern with the GCA a wide variety of prey items including testate species (Protozoa). In contrast, two detritivores (harpacticoids, non-predatory chironomids) and the predatory/omnivorous cyclopoids showed wider ranges in $\delta^{15}N$ values suggesting more than one trophic level. Cyclopoid copepods are known to feed on members of the microbial loop (Reiss and Schmid-Araya 2011), while non-predatory chironomids mostly of the genus *Corynoneura* were bacterivorous (Schmid and Schmid-Araya 1997).

Our mean $\delta^{15}N$ enrichment value was $1.896 \pm 0.157‰$, contrasting with the standard 3.4‰ fractionation of $\delta^{15}N$ (Post 2002) and being much closer to the 2.3‰ of McCutchan et al. (2003). Estimates of trophic levels depended on which fractionation standard was used, ranging between 1.75 and 2.33 (using 3.4‰) and between 1.02 and 3.76 (using 2.3‰). These values are consistent with our estimates of average chain length, but not with trophic levels based on GCA. Although trophic enrichment is crucial to estimate consumer trophic level,

there is no consensus on which of the many factors (i.e., developmental stage, taxon, feeding habits, seasons) affects $\delta^{15}N$ fractionation (Perkins et al. 2014).

Although we depicted interspecies variability in terms of the extent of $\delta^{15}N$ and $\delta^{13}C$ signature ranges, a drawback of our coarser taxonomic SIA results that resource use may be highly species specific (Vafeiadou et al. 2014). As we might have lumped species of nematodes possibly belonging to different trophic levels, the SIA may slightly underestimate chain length.

Caveats

Our data confirms recent findings such as Majdi et al. (2012) that streams have complex feeding interactions not solely depending on allochthonous organic matter but also on autochthonous resources. Our results from GCA of macro- and meiofaunal species demonstrate a strong dependence on allochthonous and autochthonous carbon sources. The SIA confirmed the latter result and placed meiofauna taxa into several trophic levels, but clearly delimiting a high trophic level for predatory chironomids.

However, some differences arose because some taxa (i.e., nematodes) were lumped for the SIA. It is well established that predatory marine nematodes can obtain their carbon through heterotrophic protists or other small prey, which in turn depend on various food sources (Moens et al. 2002). In the Lambourn, no study has assessed the abundances of ciliates, flagellates and amoeba but we speculate that they will be high (J. M. Schmid-Araya, *unpublished data*). Our results of gut content data revealed a high incidence of basal resources in the guts of predatory taxa, which would reduce $\delta^{15}N$ enrichment of predators relative to their prey, therefore, confounding isotopic analysis of trophic structure.

Obviously, feeding interactions cannot be established in GCA in piercers and suckers (i.e., nematodes, tardigrades) or those species feeding on soft-bodied taxa (i.e., gastrotrichs, microturbellarians) and most members of the microbial loop. This contrasts to benthic rotifers and testates, which are easily identifiable (Schmid and Schmid-Araya 1997).

Studies on biodiversity, food web topology, and conceptual ecological models should be further refined to include meiofauna species assemblages in order to enhance their realism, and consequently, predictive capabilities and rigor.

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