

## There's no harm in having too much: A comprehensive toolbox of methods in trophic ecology

Majdi Nabil <sup>1,2,3</sup>, Hette-Tronquart Nicolas <sup>1,4</sup>, Auclair Etienne <sup>1,5</sup>, Bec Alexandre <sup>1,6</sup>, Chouvelon Tiphaine <sup>1,7</sup>, Cognie Bruno <sup>1,9</sup>, Danger Michael <sup>1,8</sup>, Decottignies Priscilla <sup>1,9</sup>, Dessier Aurélie <sup>1,10</sup>, Desvillettes Christian <sup>1,6</sup>, Dubois Stanislas <sup>1,11</sup>, Dupuy Christine <sup>1,10</sup>, Fritsch Clémentine <sup>1,12</sup>, Gaucherel Cédric <sup>1,13</sup>, Hedde Mickaël <sup>1,14</sup>, Jabot Franck <sup>1,15</sup>, Lefebvre Sebastien <sup>1,16,17</sup>, Marzloff Martin <sup>1,11</sup>, Pey Benjamin <sup>1,3</sup>, Peyrard Nathalie <sup>1,5</sup>, Powolny Thibaut <sup>1,12</sup>, Sabbadin Régis <sup>1,5</sup>, Thébault Elisa <sup>1,18</sup>, Perga Marie-Elodie <sup>1,19</sup>

<sup>1</sup> GRET (Groupe de Recherche en Ecologie Trophique), GDR 3716 CNRS INEE INRA, France

<sup>2</sup> Universität Bielefeld, Abteilung Tierökologie, Konsequenz 45, 33615 Bielefeld, Germany

<sup>3</sup> Université de Toulouse, EcoLab, UMR 5245 CNRS, INP, UPS, ENSAT, 118 route de Narbonne, 31062 Toulouse, France

<sup>4</sup> Irstea, UR HYCAR, 1 rue Pierre Gilles-de-Gennes, 92160 Antony, France

<sup>5</sup> INRA – MIAT, UR 875, INRA, 24, Chemin de Borde Rouge, 31320 Castanet-Tolosan, France

<sup>6</sup> Université Clermont Auvergne, CNRS, LMGE, 1 impasse Amélie Murat, 63178 Aubière, France

<sup>7</sup> IFREMER – BE, LBCM, Centre Atlantique, rue de l'île d'Yeu, 44311 Nantes, France

<sup>8</sup> Université de Lorraine, LIEC, UMR 7360 CNRS, rue Claude Bernard, 57070 Metz, France

<sup>9</sup> Université de Nantes, IUML Mer-Molécules-Santé (MMS), UMR 3473 CNRS, 2 rue de la Houssinière, 44322 Nantes, France

<sup>10</sup> Université de La Rochelle, LIENSs, UMR 7266 CNRS, 2 rue Olympe de Gouges, 17000 La Rochelle, France

<sup>11</sup> IFREMER – DYNECO, LEBCO, Centre de Bretagne, CS 10070, 29 280 Plouzané, France

<sup>12</sup> Université Bourgogne Franche-Comté, Chrono-environnement, UMR 6249 CNRS, INRA, 16 route de Gray, 25030 Besançon, France

<sup>13</sup> Université Montpellier, AMAP - INRA, CIRAD, CNRS, IRD, Montpellier, France

<sup>14</sup> INRA – Eco&Sols, UMR 1222, 2 Place Viala, 34060 Montpellier, France

<sup>15</sup> Irstea, UR LISC, Campus des Cézeaux, 9 avenue Blaise Pascal, 63178 Aubière, France

<sup>16</sup> Université de Lille, LOG, UMR 8187 CNRS, ULCO, 28 Avenue Foch, 62930 Wimereux, France

<sup>17</sup> IFREMER – Laboratoire Ressources Halieutiques, 150 Quai Gambetta, 62321 Boulogne-sur-mer, France

<sup>18</sup> Université Pierre et Marie Curie, Institute of Ecology and Environmental Sciences, CNRS, Sorbonne Universités, Paris, France

<sup>19</sup> University of Lausanne, Institute of Earth surface Dynamics, Lausanne, Switzerland

### Abstract :

Trophic ecology is the study of feeding interactions and food acquisition by organisms. It includes the causes and consequences of those behaviours at all levels of biological organisation. As a field of research, it crosses many disciplinary boundaries and provides knowledge that is pertinent to many other areas of ecology. Here we list and categorise the methods available to trophic ecologists whose toolbox

---

has broadened considerably in recent years. They encompass empirical and numerical approaches with focus ranging from molecules to ecosystems. We further examine the relationship of each method to features such as the scale of observation (from microbes to largest organisms) and organisational level (from individuals to ecosystems) as well as the ecological question the method is capable of answering (from detecting predator-prey relationships to studying implications and consequences at different scales). Our survey reveals a very wide range of methodologies, each more-or-less appropriate for a particular line of research. It also identifies deficits, for example, trophic interactions at microscopic scales, for which empirical methods have hardly been used, as well as trophic models that have failed to consider fluxes at the ecosystem scale. Furthermore, we note that the combination of methodologies remains under-exploited despite great opportunities to solve complex ecological questions and to foster the emergence of new insights and hypotheses regarding organism, population and/or ecosystem properties.

**Keywords** : Food web, Feeding interactions, Flux of energy, Computer simulations, Trophic models

## 24 Introduction

25 Trophic ecology covers the ecological implications of feeding. As such, it embraces the act of  
26 food acquisition and its consequences at the individual, population and community levels up to  
27 functional outputs at the scale of the whole ecosystem. The field of trophic ecology, in which the  
28 concept of food web is embedded, is broader than organismal detection and the description of  
29 inter-species feeding relationships. Briefly, at the smallest organizational levels, feeding is  
30 intimately linked to nutrition and ecophysiology, as the quantity and quality of mineral or  
31 organic food resources regulates individual survival, growth and fecundity with upscaling effects  
32 on population demography (Elser et al., 1996). Except for lithotrophic and saprophytic  
33 organisms, food acquisition also implies that feeding interactions generate reciprocal effects on  
34 predator and prey demography (Lotka, 1925 – Volterra, 1926). Trophic relationships are also  
35 responsible for species inter-dependencies and contribute to shaping behaviours, driving  
36 phenotype selection and eco-evolutionary feedbacks (Post and Palkovacs 2009, Clinchy et al.  
37 2013). A trophic transition can also be biogeochemically regarded as a way to transfer mineral or  
38 organic matter between two trophic levels with more-or-less efficiency (“trophic efficiency  
39 concept” of Lindeman 1942, “ecological stoichiometry” see e.g. Elser et al., 1996), or  
40 transformations in its biochemical quality (“trophic upgrading”: Klein-Breteler et al., 1999). The  
41 concept of food web brings trophic ecology to the community and ecosystem scales. Food webs  
42 allows exploring the network of consumer-resource interactions, considering its assemblage rules  
43 and interaction strength and the consequences of network structure to overall community  
44 composition and stability, up to emergent properties of the ecosystem — like carbon fixation  
45 (see Layman et al., 2015, and references therein for a synthesis of major concept achievements in  
46 food web ecology).

47           The broadness of trophic ecology implies a diversity of methodologies, along with considerable  
48 improvements and developments both in field and laboratory methods. In addition, numerical  
49 approaches have flourished and now support trophic ecologists in their efforts to conceptualise their  
50 theories and to modify them based on empirical data (e.g. Proulx et al., 2005; D’Alelio et al., 2016).  
51 Considering this large and fast-evolving bouquet of methodologies, we felt that an up-to-date overview  
52 of existing methods, merging both empirical and numerical techniques, has been so far missing. Here, by  
53 providing a technical overview, we hope to facilitate both upstream decision-making and the inclusion  
54 of a broader diversity of research paths within otherwise well-defined methodological road maps.  
55 Nevertheless, we do not pretend to provide either an exhaustive critical review of every method  
56 relevant to trophic ecology or systematic pairwise comparisons of all methods. For this, we refer  
57 readers to the comprehensive book by Garvey and Whiles (2017) and to more specific methodological  
58 reviews referenced hereafter. Because the choice of a method primarily depends on its ability to  
59 answer a question at hand, we have structured this review accordingly, grouping the methodologies  
60 into four categories based on the trophic ecology question they are most suitable for addressing: (1)  
61 empirical detection of predator-prey relationships or food sources, (2) connection of species within  
62 networks based on empirical data (inference methods), (3) quantification of the consequences of  
63 feeding, from individuals to ecosystems and (4) testing the implications of trophic interactions (trophic  
64 models). For each method, we briefly describe the aims and principles, the advantages and drawbacks,  
65 and we include estimates of human and financial costs, as time and money are often decisive in the  
66 final choice. Beyond providing a methodological toolbox that will help identify the most appropriate  
67 tool (Section 5), we seek to encourage an integrated research strategy that combines approaches  
68 (Section 6) and to highlight the conceptual barriers (Section 7) in trophic ecology.

## 69 1. Detecting predator-prey relationships or food sources

### 70 1.1. Observations of feeding activity

71 The most straightforward and oldest approach to study feeding interactions relies on observations.  
72 Feeding observations can be made with the naked eye and simple equipment (e.g., binoculars) or using  
73 sophisticated, miniaturised, high-magnification optical devices, including remote underwater video  
74 stations, camera traps and drones (e.g. Linchant et al., 2015). This allows large-scale records of feeding  
75 behaviours in remote ecosystems that are poorly studied. In addition, animal-borne video systems offer  
76 the animal's perspective (e.g. Moll et al., 2007). For microscopic or suspension-feeding animals, most  
77 observations are restricted to laboratory conditions, for example using a camera attached to a  
78 microscope (e.g. Dupuy et al., 2010) or by *in vivo* video endoscopy (e.g. Prasetya et al., 2017). To  
79 complement direct observations, an emerging approach consists in remotely monitoring multiple  
80 biological and environmental factors that constitute surrogates for feeding activity, such as the  
81 movements of body parts, oesophageal or gastric temperatures and geographical positions, using  
82 animal-attached bio-logging devices (Ropert-Coudert and Wilson, 2005). This should further progress  
83 with the transfer of geo-referenced human mobility data analysis techniques to animals (Thums et al.,  
84 2018). If direct observation of feeding activity is impracticable, food intake can be inferred from the  
85 disappearance of food items. For example, suspension-feeder activity is most commonly assessed  
86 using food-clearance experiments or flow-through chamber methods (see e.g. Ward and Shumway,  
87 2004). Different parameters can be obtained this way, such as clearance rate, retention and sorting  
88 efficiencies, ingestion, and egestion rates.

89

90

91           1.2. Gut content and faeces analysis

92   Observing the food remains in digestive tracts of animals is the most common approach to get a basic,  
93   timely knowledge of predator-prey relationships. Some studies have used massive gut contents datasets  
94   to evaluate and compare trophic networks in different ecosystems (e.g. Winemiller, 1990). Especially  
95   in fishes, typical methodologies and indices have been developed, and some like the percentage of prey  
96   occurrence may emerge as relatively reliable proxies of diet composition (see Baker et al., 2014).  
97   Rejection pellets and faeces can also be collected and inspected for undigested prey items (e.g.  
98   Kusmer, 1990). In most cases, a dietary assessment is based on the recovery of hard/recalcitrant prey  
99   parts (e.g. bones, fur, diatom frustules), which makes a reference collection of such parts often  
100   necessary to identify the respective prey organism.

101           The main advantages of this type of dietary analysis consist in: (1) being accessible, (2) being  
102   applicable at relatively large spatial and temporal scales and (3) enabling assessments of prey size (e.g.  
103   measuring otoliths or vertebrae). Amongst the main drawbacks are (1) the discrepancies in taxonomic  
104   distinctness (as the ingested items can be identified either at the species level or at a higher taxonomic  
105   level), (2) the inability to account for labile food items and (3) the requirement of comprehensive  
106   taxonomic expertise. (4) Ethical issues are also raised by this approach, particularly when animals must  
107   be dissected. Alternative techniques that avoid dissection can be used to recover stomach contents,  
108   such as stomach flushing or regurgitation (e.g. Pierce and Boyle, 1991, Legler and Sullivan, 1979 ;  
109   Duffy and Jackson, 1986). In addition, as many microscopic animals have transparent bodies, their gut  
110   contents can often be examined under the microscope without dissection. For example, confocal laser  
111   scanning microscopy can be used to detect the auto-fluorescence of micro-algal pigments in the gut of  
112   rotifers (Mialet et al., 2013), and chromatography to quantify ingested biomarker pigments extracted  
113   from guts (e.g. Majdi et al., 2012).

114 Molecular approaches can also be used to decipher ingested prey remains based on the use of  
115 specific prey primers to amplify the prey DNA contained in the gut or faeces of the predator followed  
116 by (meta)barcoding (for a review see King et al., 2008). The use of molecular gut content has really  
117 expanded in recent years and has proved useful to assess, for instance, the diet of minute animals such  
118 as soil micro- and meso-arthropods (e.g. Eitzinger et al. 2013; Heidemann et al. 2014), and  
119 zooplankton (e.g. Ho et al. 2017). The major advantages of analysing gut content DNA are: (1) high  
120 taxonomic resolution without taxonomic expertise, (2) the inclusion of partly digested, unidentifiable  
121 prey chunks and, potentially, (3) the acquisition of data on the microbial assemblages ingested or  
122 persisting in the gut (i.e. gut microbiomes, see, e.g. Derycke et al., 2016). However, besides substantial  
123 costs, there are several disadvantages inherent to molecular approaches, especially (1) high risk of  
124 sample contamination and false positives, (2) need for specific primers to identify uncommon prey  
125 taxa, (3) absence in public gene repositories of sequence data for the DNA of numerous prey types, (4)  
126 bias in quantitative extrapolations due to the differential timing of DNA degradation during digestion  
127 or the specificity of certain DNA extraction protocols and (5) the inability to detect cannibalistic  
128 behaviour (Traugott et al., 2013).

129

### 130 1.3. Parasites as biological tags

131 Since some parasites are transmitted only through food ingestion, they serve as evidence of a feeding  
132 interaction and indicate that the host species has fed in geographical areas where the prevalence of this  
133 parasite is high. Similarly, accurate information about the feeding grounds and past movements of  
134 animal hosts can be surmised based on the presence of multiple parasite species differing in their  
135 endemic areas (MacKenzie and Abaunza, 1998). Yet, to be reliable biological tags, parasites must  
136 have: (1) distinct prevalences in different studied areas, (2) limited inter-annual or seasonal variations

137 in those prevalences, (3) a high specificity for the microhabitat within the host's body, (4) a lifespan  
138 fitting the aim/scale of the study, (5) no strong pathological effects on the host and (6) preferably, a  
139 direct and single-host life cycle (facilitating interpretations). They must also be readily detectable and  
140 identifiable. Helminths generally fulfil all of these criteria and are thus the most commonly used  
141 trophic tracers (see Mackenzie, 2002).

142         The use of parasites as trophic tracers has thus far been limited to marine ecosystems, but,  
143 given the ubiquity of parasites (Marcogliese, 2004), implementation of this method in other ecosystems  
144 is certainly possible (see Holmstad et al., 2004). Additional advantages of parasites are that they  
145 provide information even when their host's stomach is empty (Baldwin et al., 2008) and indicate long-  
146 term feeding, as they can accumulate in their hosts for months or longer (Marcogliese and Cone 1997).  
147 However, this method also requires that the animals are killed and dissected, the method is time-  
148 consuming and considerable taxonomic expertise is required. Some of these shortcomings might be  
149 overcome using DNA barcoding, which is inexpensive (~5€ /parasite), or by coupling behavioural and  
150 molecular approaches (Poulin, 2010). However, the use of a parasite as a biological tag relies on an at  
151 least partial understanding of the parasite's life cycle, which probably explains their use mostly in fish  
152 species so far.

153

#### 154         1.4. Tracers of biomass

155 Trophic tracers of biomass origin are elements or molecules that an organism can gain only from its  
156 trophic resources. The presence of a tracer in the biomass of an organism indicates the consumption  
157 and assimilation of a resource containing that tracer, as well as whether and how much the different  
158 trophic resources have contributed to the biomass of the organism (or its targeted tissue), provided that  
159 the potential resources differ in their tracer composition. Moreover, the presence of a tracer not only



160 reflects the consumption of a resource, it can also provide an information on how the tracer is retained  
161 and transferred within the consumer, such as by selective digestion or assimilation (Gannes et al. 1997)  
162 or routing to different tissues (Carleton et al. 2008).

163

#### 164 *1.4.1. Stable isotope composition of bulk tissues*

165 One of the most common trophic-tracer-based methods is the determination of the stable isotope  
166 composition of bulk tissues, such as muscle, blood, liver, feather, hair, fin, or whole organism. In  
167 trophic ecology, C, N and S are the tracers most frequently used in stable isotopic analysis (SIA) (Fry,  
168 2006), but H/D and O isotopes are also employed (Vander Zanden et al., 2016). The method is based  
169 on predictable differences (trophic discrimination factors, TDFs) between the isotopic composition of  
170 an organism and that of its food resources. For C and S, the difference is limited and these elements are  
171 mainly used to trace the origins of the food resources from which the consumers' biomass has been  
172 synthesized (Boecklen et al., 2011), whereas the TDF for N is larger, allowing its use in estimates of  
173 trophic position (Post, 2002). However, the variability in the TDFs depending on environments, trophic  
174 levels, taxa, tissues, etc. (Bond and Diamond 2011) is a potential confounding factor, that has been  
175 highlighted in several studies (e.g. Bastos et al. 2017). In addition, the high variability of stable isotope  
176 compositions in space and time (e.g. Hyndes et al. 2013) must also be considered. Several tools are  
177 used to analyse stable isotope data. Their most recent versions generally adopt a similar Bayesian  
178 approach to account for the sources of uncertainty (e.g. Parnell et al., 2013) and include associated  
179 confidence intervals. Bayesian approaches also allow introducing informative priors, increasing the  
180 accuracy and precision of the estimates. We distinguished three main types of tools: (1) those devoted  
181 to the estimation of trophic position (e.g. tRophicPosition, Quezada-Romegialli et al. 2018), (2)  
182 isotopic metrics to measure specimen or species positions in the isotopic space and to compare the

183 variability of isotopic signatures across individuals, populations and communities (SIBER, Jackson et  
184 al. 2011; Cucherousset and Villegger 2015) and (3) mixing models to estimate the contribution of each  
185 potential trophic resource to the biomass of an organism (see Philips 2012 for a review). The relevance  
186 of SIA-derived results is thus strongly tied to isotopic discrimination between resources, the spatial and  
187 temporal variability of the isotopic compositions captured by resource/consumer sampling and an  
188 accurate estimate of the TDF. SIA is not well suited to situations involving large numbers of potential  
189 resources (e.g. Robinson et al. 2018). The integration of the food isotopic signal into the consumer's  
190 tissue depends of the rate at which tissue are renewed, by somatic growth or metabolic tissue  
191 replacement (which determines isotopic turnover). Issues related to isotopic turnover thereby limit the  
192 relevance of the method for individuals (i.e. adults) or seasons of reduced somatic growth (gonadic  
193 growth or maintenance metabolism, Perga and Gerdeaux, 2005). Issues related to differential isotopic  
194 routing (Gannes et al., 1998, Del Rio et al. 2009) and unequal TDF in plant *versus* animal food sources  
195 (Perga and Grey 2010) also limit the detection of omnivory for individuals and species exploiting both  
196 plant and animal resources. As a result, SIA enables quantitative estimates of elemental fluxes within  
197 the different steps and habitats of a food web, but it is a less than ideal method with which to assess the  
198 diversity and specificity of a consumer's diet.

199       When natural isotope abundance does not provide the necessary discrimination between sources,  
200 for example, to overcome uncertainties linked to TDF variability, small quantities of isotopically  
201 labelled sources (stable or radioactive) can be injected *in-* or *ex-situ* to track C and/or N pathways. This  
202 method of isotope labelling, or Stable Isotope Probing (SIP) aims to track the integration of the  
203 labelled elements within food webs (Pace et al., 2007). Primary producers can be selectively labelled  
204 through addition of  $^{13}\text{C}$ -enriched bicarbonate/carbon dioxide or  $^{15}\text{N}$ -enriched ammonium or nitrate  
205 directly *in situ* (see e.g. Middelburg et al., 2000) to track the fate of primary production through food

206 webs. Similarly, SIP based on dissolved organic matter (e.g. Hall and Meyer, 1998), phytodetritus (e.g.  
207 Scharnweber et al. 2014) or animal feces (e.g. Dungait et al., 2009) are used to label microbial  
208 decomposers and the fate of dead organic matter through “brown” food webs. The mode (single  
209 addition, pulse chase, or constant levels) and the duration (a few hours to several months) at which the  
210 label is introduced into the system determine the time and scale at which the fate of the labelled  
211 substrate is tracked (from microbial interactions up to the whole ecosystem), and eventually to  
212 document the dynamics of the processes (when performed in pulse chase) (e.g. Maxfield et al., 2012).  
213 If studies using isotope labelling are relieved from the issues of TDF uncertainties encountered for SIA  
214 in natural abundances, they also suffer for issues related to isotope routing. Indeed, structural  
215 components of plant material behave differently than metabolic components in terms of microbial  
216 utilisation, grazing, and assimilation (see e.g. Soong et al., 2014). The success of isotope-labelling  
217 experiments is thereby strongly tied to the ability to uniformly label throughout the food source, or  
218 instead to differentially label the structural and metabolic components of the source (Soong et al,  
219 2014). SIP studies have proven powerful in documenting microbial involvement in biogeochemical  
220 processes in soils and sediments and in quantifying the trophic links between the microbial and macro-  
221 faunal worlds (e.g. Crotty et al. 2012; Middelburg, 2014).

222

#### 223 *1.4.2. Fatty acid trophic biomarkers*

224 Since the early 1980s, fatty acids (FAs) have been widely used to study predator-prey relationships and  
225 to trace the origin and transfer of organic matter in food webs (St-John and Lund, 1996). The use of  
226 FAs as trophic markers relies on the specific FA patterns exhibited by basal resources that are then  
227 transferred to and identifiable in consumers. As such, FAs have been analysed in many species to infer  
228 feeding relationships in aquatic environments for example (Arts et al., 2009). They are increasingly

229 used to shed light on feeding channels in soil environments (e.g. Ruess et al., 2007). Most often, this  
230 method requires tracing specific FAs of prey that cannot be synthesized *de novo* by the targeted  
231 community of consumers/predators (e.g. 16:4 $\omega$ 3 for chlorophytes, 16:4 $\omega$ 1 for diatoms, 22:1 $\omega$ 11  
232 biomarker for Calanoid copepods). The detection of those prey-specific FAs in the tissues of  
233 consumers/predators thus reveals the assimilation pathway of the prey. However, many fatty acids (e.g.  
234 16:0, 18:0, 18:3 $\omega$ 3, 18:2 $\omega$ 6) occur in a wide range of organisms restraining the accuracy of the  
235 approach (Dalsgaard et al., 2003). For this matter, the comparison of FA ratios can improve the  
236 interpretation of results (Parrish, 2003). For example tracking the 16:1 $\omega$ 7/16:0 ratio for diatoms or the  
237 22:6 $\omega$ 3/20:5 $\omega$ 3 ratio for chrysophytes can be used to demonstrate that these microalgae are assimilated  
238 by zooplanktonic microcrustaceans (Desvillettes et al., 1997; Pepin et al., 2011). Indeed,  
239 the reliability of FA-based trophic markers requires in-depth knowledge of the lipid metabolism of  
240 both primary producers and the targeted animal species as a prerequisite. However, while these  
241 metabolic processes may obscure the identification of actual food resources, FA-based methods  
242 generally provide sufficient information on foraging patterns.

243 To date, most studies using FA have been qualitative with the presence/absence of a  
244 source-specific FA being used as an indicator of the existence of a trophic interaction between  
245 this source and the consumer under study. A Quantitative FA Signature Analysis (QFASA) has  
246 been more recently developed to enable the estimation of the proportions of different sources in a  
247 predator's diet (Iverson et al. 2004). Basically, the FA signatures of consumers are modelled as a  
248 mixture of the FA signatures of major food resources, which provides a quantitative estimate of  
249 the proportions of those resources. The method is based on the calculation of the statistical  
250 distance between predator FA signatures and mean prey FA signatures. The predator's FA  
251 patterns are corrected according to the degree of modification linked to its FA metabolism.

252 Calibration coefficients are then necessary and calculated using experimental diet studies. These  
253 studies are designed to assess the selective retention of dietary fatty acids, to detect fatty acid  
254 bioconversion or *de novo* synthesis. The acceptance of this method by researchers studying the  
255 trophic ecology of marine mammals for example will likely be broadened by the recent  
256 development of a dedicated R-package (Bromaghin, 2017). According to Happel et al. (2016),  
257 however, as for mixing models in SIA, the use of QFASA should be limited to the predators that  
258 consume a small number of species and that can be studied experimentally as well as in the wild.

259

#### 260 *1.4.3. Compound specific isotopic analyses (CSIA)*

261 Combining detection of biomarkers (lipids and amino-acids mainly, but also amino-sugars) to stable  
262 isotope analyses, CSIA is able to trace the sources and pathways of numerous natural as well as  
263 anthropogenic organic compounds. In ecology, first utilizations of CSIA focused on chemotaxonomic  
264 markers of microorganisms. Although the role of microorganisms is crucial in processing and  
265 transferring organic matter in food webs, their isotopic signatures were rarely reported due to the  
266 difficulty of isolating specific microbial biomass from natural samples. Using  $^{13}\text{C}$  labelled substrates  
267 Boschker et al. (1998) described a CSIA approach to directly link specific microbial processes with the  
268 organism involved, based on the stable carbon isotope labelling of individual lipid biomarkers. In the  
269 same way, Werne et al. (2002) followed the  $^{13}\text{C}$  depleted signal of methane in cold seep sediment  
270 through different specific microbial markers and could trace with smart elegance the flow of methane-  
271 derived-carbon through anaerobic methane-oxidizing archaea (archeol and sn-3-hydroxyarchaeol) into  
272 sulfate-reducing bacteria (C15 and C17 iso and anteiso fatty acids, dialkyl glycerol diethers), as well as  
273 into aerobic methane-oxidizing bacteria (diploptene, diplopterol) up to bacterivorous ciliates  
274 (tetrahymanol). Stable isotope probing of amino-sugars is regarded as a promising tool to investigate

275 fungal-bacterial interactions in soils (Bodé et al, 2013). Thus, CSIA can be used successfully for  
276 elucidating source and fate of naturally or artificially labelled substrates.

277 In other cases, the isotopic fractionation between substrates and chemotaxonomic markers must  
278 be known. Food webs studies have thus applied CSIA on essential lipid compounds such PUFA or  
279 sterols by assuming that TDFs would be negligible. However, an unpredictable fractionation ranging  
280 from 0‰ to 4‰ still occurs during trophic transfer of essential lipid compounds such sterols  
281 (Chamberlain et al., 2004) or PUFA (Bec et al., 2011, Gladyshev et al., 2016). Moreover, the  
282 intermolecular variability of  $\delta^{13}\text{C}$  values within the same sample may be relatively high, thereby  
283 challenging again data interpretation. For example, the difference between fatty acids  $\delta^{13}\text{C}$  values  
284 within a single cultivated alga may be up to 7.6‰ (Schouten et al., 1998). Thus, interpretation of CSIA  
285 data could be constrained in case the potential endmembers are not sufficiently isotopically different.  
286 However, when the conditions are met, CSIA could trace limiting compounds and eventually detect  
287 minor food sources unseen by classical methods. For example, CSIA has revealed an uncoupling  
288 between essential compounds and major organic matter transfers highlighting functional importance of  
289 minor food sources of great nutritional importance (Koussoroplis et al., 2010). An increasing attention  
290 is now being paid to amino acids-CSIA since McClelland and Montoya (2002) showed that the  $\delta^{15}\text{N}$   
291 values of some amino acids such phenylalanine in consumers are very similar to those in the producers  
292 while the  $\delta^{15}\text{N}$  values of other amino acids such glutamic acid become enriched in  $^{15}\text{N}$  with each  
293 trophic transfer. Thus, due to this differential  $^{15}\text{N}$  enrichment with trophic transfer,  $\delta^{15}\text{N}$  of amino acids  
294 in a consumer provide a measurement of the  $\delta^{15}\text{N}$  baseline as well as its trophic position.

295 Coupled with stable isotopic probing (also coined SIP), ribosomal RNA or DNA can also be  
296 used as integrative tracers.  $^{13}\text{C}$ - or  $^{15}\text{N}$ -labelled RNA or DNA is heavier and can be separated from  
297 unlabelled material using isopycnic ultracentrifugation, for example. Further amplification and

298 barcoding of the isolated fractions allows an organism associated with the assimilation of a specific  
299 resource or organic contaminant to be identified (Neufeld et al., 2007). In microbial ecology, this is a  
300 promising approach to detect specific functional guilds of microbes (e.g. methanotrophic bacteria), but  
301 it can also be scaled-up to other consumers, such as fungi and flagellates, to reveal matter flows in  
302 microbial food webs (Lueders et al., 2003).

303

#### 304 *1.4.4. Contaminants as trophic tracers*

305 Both trace metals (inorganic elements) and xenobiotic organic compounds can fulfil the role of trophic  
306 tracers (Ramos and González-Solís, 2011) when these contaminants enter trophic pathways and  
307 differentially bio-accumulate in food sources or prey and consequently in consumers or top predators  
308 (e.g. Walters et al., 2008; Pitt et al., 2017). Selected contaminants can thus be used to infer trophic  
309 relationships, including the prey preferences of consumers/predators, foraging areas or food webs  
310 exploited by different individuals or populations (e.g., Larsson et al., 1990; Deshpande et al., 2016)  
311 and even trophic positions and organic matter flux when bio-magnification occurs (Kelly et al., 2007).  
312 Therefore, these indirect tracers can be particularly helpful when there is inherent difficulty of direct  
313 observations of trophic relationships (e.g., for marine species). Another advantage of using  
314 contaminants in a trophic perspective is that it also informs on the contamination status of the species  
315 and food webs of concern, which may be very interesting for ecotoxicological and/or conservation  
316 biology perspectives as well. For example, a survey of cadmium (Cd) in common dolphins from the  
317 North-East Atlantic allowed neritic and oceanic populations to be distinguished based on the Cd  
318 concentrations in their kidneys (long-term accumulation organ in mammals) and their distinct  
319 preferential prey (Lahaye et al., 2005). Cephalopods and especially oceanic squids (i.e. the preferential  
320 prey of oceanic dolphins compared to neritic ones) indeed display very high burden of this metal

321 relative to fish prey species, and were thus proved to constitute a significant vector of Cd for their  
322 predators (Caurant and Amiard-Triquet, 1995; Bustamante et al., 1998).

323         However, while monitoring contaminants can yield information on both trophic interactions  
324 and matter fluxes, good knowledge of the contaminants' characteristics (biogeochemical cycles, the  
325 distribution and persistence of the contaminant in organisms and the environment), the target  
326 organism's biology, and the structure of food webs are essential. Another prerequisite is an obvious  
327 source of the contaminant and its clear partitioning. In fact, contaminants are rarely used alone as  
328 trophic tracer tools, and rather complement other intrinsic trophic markers such as C, N, S isotopic  
329 ratios or FA profiles (e.g., Krahn et al., 2007; Hebert et al., 2009; Praca et al., 2011). For example, in  
330 prey with similar FA but different contaminant profiles, use of the latter allows the discrimination of  
331 different diets in the predator (while the use of FA alone did not). Nevertheless, the use of  
332 contaminants as trophic tracers requires that the analysed tissues be carefully chosen, because the type  
333 of tissue strongly influences the turnover rate of the contaminant-containing compound, which in turn  
334 modifies the integration time of the contaminant itself. Finally, like parasites-based approaches, the  
335 toxic effects of a contaminant may alter the feeding strategy of the organism. However, as long as the  
336 contaminants are able to track these potentially altered foraging behaviours, they fulfil their role as  
337 trophic tracers. With the recent development and improvement of modelling tools such as the  
338 "Ecotracer" module (Walters and Christensen, 2018) in the Ecopath with Ecosim software package  
339 (see Sections 2.3 and 4.1), the potential of contaminants as trophic tracers could be further extended.

340

341

342



## 343 2. Connecting species within networks from ecological data (inference methods)

344 Because establishing complete inventories of trophic interactions within a system is virtually  
345 impossible, inference methods can be used to reconstruct trophic networks when the ecological  
346 datasets are incomplete (Jordano, 2016). Inference methods are thus specifically useful when empirical  
347 approaches (such as those described in section 1) cannot be implemented to assess trophic interactions.  
348 In that case, inference methods combine empirical data about the community composition (mostly  
349 occurrence/abundance of the different species) and theoretical knowledge about trophic ecology (e.g.  
350 trophic behaviour of the species) to infer the trophic interactions within the community. It is worth  
351 noting that inference methods were not specifically developed for trophic interactions, but for all kinds  
352 of interactions (from causal relationships among variables, to social networks). Several ways are  
353 available to infer interactions (e.g. using Gaussian distributions, logical interaction rules or likelihood  
354 postulates), each leading to a different method that can be applied to trophic interactions. In the  
355 following, we only focus on the inference methods that are mainly used in the reconstruction of trophic  
356 networks.

357

### 358 2.1. Graphical models

359 A Graphical model is a probabilistic model using a graph to represent the dependencies among variables.  
360 In the case of trophic networks, the variables are species abundances or occurrences and the edges in  
361 the graph can be interpreted as trophic interactions. Generally, the graph structure is given by expert  
362 knowledge, and graphical models are used to make predictions about species persistence. Conversely,  
363 it is also possible to learn the graph structure, in our case the trophic network, using empirical data.  
364 The principle is to draw an edge between two species in the community, whenever their  
365 abundances/occurrences are not independent. Two main kinds of graphical models are used in trophic

366 ecology: Gaussian Graphical Models (GGMs) (Højsgaard et al., 2012) and Bayesian Networks (BNs)  
367 (Jensen and Nielsen, 2007).

368 With GGMs, independence between two species is assessed from the empirical data with the  
369 precision matrix (i.e. the inverse of the covariance matrix of the model). The resulting network is an  
370 undirected interaction network whose edges are weighted by the coefficient of the precision matrix.  
371 Expert knowledge is then required to determine the nature and the direction of the inferred interactions  
372 (trophic or non-trophic links). In addition, it is often necessary to limit the number of inferred  
373 interactions to the most relevant ones. To do so, GGMs are coupled with sparse regularisation  
374 techniques, such as the graphical LASSO (for Least Absolute Shrinkage and Selection Operator;  
375 Friedman et al., 2008). Faisal et al. (2010) tested different GGM procedures on large-scale spatial data  
376 sets describing abundance records for 39 species of European Warblers. They were able to reconstruct  
377 *in silico* the ecological networks, and from the inferred networks, they could compare the relative role  
378 of bioclimatic *versus* biotic interactions on the species spatial distribution.

379 Unlike GGMs, BNs and their temporally explicit extension, dynamic-BNs (Dean and Kanazawa,  
380 1989) rely on directed graphs. Concretely, it means that an edge can be interpreted directly as a  
381 predation link. BNs also differ from GGMs by the learning process of the graph. In the case of BNs,  
382 every possible graph is used to assess species abundances/occurrences. Then, each graph receives a  
383 score, depending on both the fit between observed and inferred abundances/occurrences and graph  
384 complexity. Scores are calculated using different statistical score functions (e.g. Bayesian information  
385 criterion), but the underlying principle is the same: to find a graph (in our case a trophic network) that  
386 maximises the score, exactly or approximately. In trophic ecology, BNs and dynamic-BNs have been  
387 successfully applied to infer feeding interactions among species based on synthetic data (Aderhold et  
388 al., 2012), by combining expert knowledge and field data, such as long-term catches per unit effort of

389 fisheries (Trifonova et al., 2015), or based on the presence-absence time series of a fish community  
390 (Sander et al., 2017).

391

## 392 2.2. Logic-based approaches

393 Logic-based approaches differ from graphical models by the way they reconstruct trophic networks.  
394 The principle is not based on statistical approaches, but on a set of logical rules (in the form of: “if”  
395 *premise* “then” *consequence*) that is used to reconstruct trophic networks. The main advantage of such  
396 approaches is to establish the rules by combining background knowledge about the species and their  
397 environment and empirical data. In trophic ecology, two techniques are commonly used to determine  
398 the set of logical rules: Inductive logic programming (Muggleton, 1991) when background knowledge  
399 is available; or meta-interpretive learning (Muggleton et al., 2014) when background knowledge is  
400 incomplete or lacking, (e.g. when the trophic ecology of the species is not known and is actually the  
401 object of the attention). Logic-based approaches have been successfull employed to automatically  
402 generate trophic networks. For instance, Bohan et al. (2011) reconstruct the trophic network of  
403 invertebrate communities within arable fields in Great Britain, using logic-based machine-learning  
404 algorithms, species occurrence data and background knowledge, such as species body-size and  
405 functional groups.

406

## 407 2.3. Linear inverse inference

408 In linear inverse inference, trophic networks are represented in a steady state, as static webs of model  
409 compartments (e.g. species or group of species) that are linked together by linear interactions (Van  
410 Oevelen et al., 2010). The approach is particularly suited to infer the magnitude of missing  
411 interactions, a very common situation in many ecosystems given the high-dimensionality of trophic  
412 networks and the scarcity of information across all trophic levels. Linear inverse inference estimates

413 the missing interactions by ensuring mass-balance across all compartments. Practically, different kinds  
414 of techniques can be used (see Van Oevelen et al., 2010) to select the set of parameters that produce  
415 the best fit between the modelled abundances (or biomasses) of each compartment and empirical  
416 observations. The Ecopath software (see Christensen and Pauly, 1992) constitutes a typical example of  
417 this approach. In addition, linear inverse inference can be coupled with ecological stoichiometry or  
418 stable isotopic analysis to further constrain the solution range of the reconstructed food webs. For  
419 instance, Pacella et al. (2013) associated isotope data with linear inverse modelling to reconstruct the  
420 feasible food webs within the Marennes-Oléron intertidal seagrass bed.

421

#### 422 2.4. Approximate Bayesian computation

423 Bayesian inference methods rely on the computation of a likelihood, which is the probability density of  
424 the observed data under the studied model. It is associated with the parameter values of the model. As  
425 a likelihood computation is not always possible, approximate Bayesian methods can be used instead,  
426 with the most common one being the Approximate Bayesian Computation (ABC). This approach  
427 originates from the field of population genetics (Tavaré et al., 1997) but it has been successfully  
428 applied in trophic ecology to decipher the impacts of trophic interactions on the spatial structure of  
429 communities (e.g. Jabot and Bascompte, 2012). In an ABC, the likelihood computation step is replaced  
430 by intensive simulations of the model with variable parameter values, so as to select the values that  
431 lead to the best match between simulations and empirical data. ABC can be further coupled to efficient  
432 algorithms, such as sequential or traditional Markov Chain Monte Carlo methods (Jabot et al., 2013).  
433 This approach was used to evidence the presence of density-dependent dispersal in spatially distributed  
434 food webs, as well as its implication for food web spatial structure (Melián et al., 2015).

435

### 436 3. Quantifying the consequences of feeding from individuals to ecosystems

#### 437 3.1. Ecophysiological markers

438 Nutritional status and/or body condition can be assessed using diverse types of information, such as  
439 foraging success or the energy stored by individuals (Schulte-Hostedde et al., 2001). Using  
440 ecophysiological approaches among others, nutritional status and body condition can be helpful to (1)  
441 infer feeding activities and foraging history of individuals and populations, and (2) the consequences of  
442 trophic relationships and availability of resources on individual health and fitness as well as population  
443 and community dynamics. Despite the existence of extremely invasive or lethal methods currently  
444 available to assess body condition, body composition and nutritional status, non-lethal techniques are  
445 increasingly used. As described in recent reviews, these techniques include body condition indices,  
446 body fluid biochemistry, chemical dilution techniques (isotope dilution: change in concentration of  
447 isotopes of hydrogen and/or oxygen in body water, and gas dilution: change in concentration of lipid  
448 soluble gas), electrical approaches such as body electrical conductivity and bioelectrical impedance,  
449 scanning methods (e.g. magnetic resonance imaging, ultrasound scanning, dual-energy X-ray  
450 absorptiometry, computed tomography), molecular analyses (e.g. gene expression) and measurements  
451 of stress hormones and immune responses (Stevenson and Woods, 2006; Wilder et al., 2016).  
452 In addition, animal performance (i.e. various life-history traits related to fitness, such as survival,  
453 development rate and growth, resistance to starvation, tests of stamina, and reproductive success) can  
454 be measured. These data are often used to assess the physical condition of the animal, or to evaluate  
455 the “quality” of its food and/or habitat and determining factors of trophic interactions or driver of diet  
456 selection (Harrison et al., 2011; Jakob et al., 1996; Raubenheimer and Simpson, 2004; Visanuvimol  
457 and Bertram, 2011). Metabolism is also a physiological indicator; it can be determined through  
458 respirometry, which measures the basal metabolic rate (at rest) or the maximum oxygen consumption

459 rate during forced exercise (Sadowska et al., 2008; Urrejola et al., 2011). Among this wide range of  
460 techniques, our focus here is on those most widely used, easy to conduct, least invasive and least  
461 expensive, namely, body condition indices and body fluid biochemistry.

462

### 463 *3.1.1. Body condition*

464 Body composition, or the amount of fat vs. fat-free body mass, is probably one of the most direct  
465 indicators of body condition. It provides a quantitative assessment of energy storage because lipid  
466 stores constitute the principal form of energy reserves in animals (Schulte-Hostedde et al., 2001;  
467 Walsberg, 1988). Thus, the body's energy reserves are measured through analyses of body fat content  
468 or body composition. The latter is mostly based on two or three compartments: fat mass, lean dry  
469 mass/water mass and protein and ash mass (Jakob et al., 1996; Peig and Green, 2009; Reynolds et al.,  
470 2009; Schulte-Hostedde et al., 2005). Alternative, but destructive methods, that can be conducted in  
471 study designs based on carcass collection for instance include the sampling and weighing of specific  
472 subcutaneous fat depots during dissection or the use of complementary somatic indices such as those of  
473 the liver or pancreas (Stevenson and Woods, 2006). However, such methods are tedious, time-  
474 consuming and intrusive as well as destructive.

475 Consequently, other methods have been proposed to measure body composition or fat stores,  
476 notably total body electrical conductivity (Reynolds et al., 2009; Walsberg, 1988), magnetic resonance  
477 imaging (McGuire and Guglielmo, 2010) and body composition modelling (Molnar et al., 2009). In  
478 certain taxonomic groups (such as birds), subcutaneous adipose depots can be assessed by simple  
479 visual inspection (Labocha and Hayes, 2012), whereas others (small mammals) require simple  
480 measurements, such as pelvic circumference, considered representative of fat content (Labocha et al.,

481 2014). In microscopic organisms, coherent anti-Stokes Raman spectroscopy can be used to non-  
482 invasively observe lipid droplets in tissues (e.g. Fueser et al., 2018).

483         Body condition indices based on morphometrics have also been developed. Most discriminate  
484 between the mass of the individual associated with body structural size (body weight) and the mass  
485 related to energy reserves (Green, 2001; Peig and Green, 2009; Schulte-Hostedde et al., 2005).  
486 Accordingly, the greater the body weight is, the larger are the energy reserve, and the better is the body  
487 condition. Body condition indices thus allow the relative size of the body's energy stores to be  
488 computed, taking into account body structural size components. Historically, this was achieved using  
489 the residuals of the regression (typically, ordinary least squares regression) between body weight and  
490 some index of body size, or by calculating the scaled mass index (Green, 2001; Peig and Green, 2009,  
491 2010; Schulte-Hostedde et al., 2005). Although much debated (Wilder et al., 2016), body condition  
492 indices based on morphometrics have been, and are still, widely applied (Labocha et al., 2014;  
493 Labocha and Hayes, 2012; Wilder et al., 2016).

494

### 495         3.1.2. *Body fluid biochemistry*

496 Several (micro-) nutrients needed to fulfil basic energetic requirements and metabolism cannot be  
497 synthesised *de novo* and can only be acquired from food (Kohl et al., 2015; McWilliams, 2011). These  
498 essential trace elements include dietary minerals (e.g. Cu, Co, Fe, Mn, Mo, Se, Zn), carotenoids,  
499 vitamins and some amino acids and FAs (Costantini et al., 2010; Harrison et al., 2011; Kohl et al.,  
500 2015; McWilliams, 2011). By measuring the levels of certain metabolites, electrolytes and enzymes,  
501 the depletion of some essential or limiting dietary element, and thus the health state (or the presence of  
502 food deficiencies) of the individual, can be determined directly. Moreover, information are given on

503 how the resource balance is oriented toward the use (and even storage) of nutrients from the acquired  
504 food or toward the mobilisation of endogenous reserves.

505         With the aid of diagnostic tools similar to those used in human and veterinary medicine, body  
506 fluid biochemistry can be used to assess body condition and individual health in other organisms  
507 (Resano-Mayor et al. 2016; Schoepf et al. 2017), especially for micro-nutriments only acquired by  
508 food. Since the late 1990s, interest in non-lethal methods to evaluate the physiological state of  
509 individuals has grown and has stimulated the development of technical devices and commercial kits  
510 compatible with the measurement of micro-volumes. Thus, biochemical analyses of plasma/serum or  
511 hemolymph can be conducted by measuring various markers related to nutrition and digestion,  
512 metabolism, nutrient storage, osmoregulation, liver or kidney function and immunity. Specifically,  
513 nutritional status and patterns of energy utilisation can be assessed by measuring the levels of the  
514 products of intermediary metabolism related to diet and detectable in body fluids. This is the case for  
515 total proteins, glucose, albumin, urea, creatinine, uric acid, essential minerals and trace elements. Lipid  
516 metabolism can be assessed by measuring the concentrations of lipids and ketones, homeostasis,  
517 energetic metabolism or the functioning of tissues involved in digestion based on the activities of  
518 specific enzymes (e.g. alkaline phosphatase, lactate dehydrogenase and creatine kinase). There are also  
519 markers specifically associated with starvation, such as bile acids, bilirubin and globulin, and those that  
520 allow accurate assessments of hepatic, renal and pancreatic function or electrolyte levels (major and  
521 dietary minerals). Analyses of body fluids may also target specific micronutrients essential for normal  
522 metabolic processes and thus potentially indicative of health status. An example is carotenoids. These  
523 fat-soluble pigments originate from plants, algae or fungi but cannot be synthesised *de novo* by  
524 vertebrates, which must acquire them exclusively from dietary sources. Hence, carotenoid-based traits



525 can reveal the foraging ability, resource availability and trophic interactions as well as the nutritional  
526 and immune status of their bearers (Sternalski et al., 2010).

527         The main drawback of these techniques lies in the difficulty of interpreting the data obtained  
528 from the battery of markers. The measured parameters must be chosen carefully to obtain convincing  
529 evidence of a metabolic pattern or change in nutritional status. Furthermore, some parameters can be  
530 interpreted only in the light of variations in others (for instance lipids or hepatic enzymes), and body  
531 fluid biochemistry can be affected by several confounding factors in the relationship with food  
532 acquisition (for instance, blood/hemolymph metabolite concentrations may change after food  
533 ingestion, and age, sex, and reproductive stage can affect metabolic levels and enzymatic activities).  
534 Given these considerations, an integrated assessment is critical and should be based on the use of  
535 several body fluid biomarkers, other metrics of body condition and could be dependant of individual  
536 and ecological factors as well (Resano-Mayor et al., 2016; Wilder et al., 2016). Changes in  
537 physiological conditions are not always food-related but may be driven by other abiotic stressors (e.g.  
538 temperature changes, contaminants) that might be considered carefully but that are important in trophic  
539 ecology as well as to get further insights into the consequences of the interactions between  
540 environmental factors and trophic relationships (see for instance Bustnes et al., 2015). Such issues are  
541 raising growing interest within the framework of stress ecology. Nutritional ecology is one side within  
542 trophic ecology framework that is perhaps not enough considered to build integrated approaches and  
543 may deserve further attention in studies on food webs and trophic ecology (Raubenheimer et al., 2009).

544

545

### 546 3.2. Trait-based trophic ecology

547 Focusing on functional features rather than on taxa helps describing biodiversity from a functional  
548 point of view (Levine, 2016). Trait-based approaches can be an adequate theoretical framework for  
549 understanding and predicting food webs dynamics and properties (Bartomeus et al., 2016; Gravel et al.,  
550 2016). Thus, trait-based trophic ecology investigates both (1) trophic interactions *per se* and (2) their  
551 ecosystem implications. First, functional traits allow inferences regarding the most probable  
552 interactions occurring within a community. A common first approach uses phenological traits or  
553 ecological preferences to detect species that co-occur and to discard unlikely trophic interactions  
554 (González-Varo and Traveset, 2016). The morphological and/or physiological traits of the co-occurring  
555 partners are then used to infer the probability of realised trophic interactions (see Gravel et al., 2016 for  
556 a useful framework distinguishing foraging, vulnerability and consumption traits). Because  
557 determining all interactions among individuals, even in simple systems, is fairly unattainable,  
558 narrowing those interactions using functional traits provides an alternative and can make the  
559 mechanistic underpinnings of interactions understandable (e.g. Ibanez et al., 2013). In addition,  
560 functional traits enable the different dimensions of the trophic niche to be investigated individually or  
561 simultaneously by examining the morphological, physiological, phenological and behavioural  
562 determinants of the trophic niche of a particular species (e.g. Spitz et al., 2014).

563 Two tenets of functional-trait-based approaches are essential in interpreting the implications of  
564 the identified trophic interactions. First, ‘response and effect traits’ link the response of individuals to  
565 higher trophic levels or to environmental stressors with the potential effects on lower trophic levels.  
566 For instance, plant diversity changes affects functional diversity of arthropods across trophic levels  
567 (Ebeling et al., 2018) which might affect ecosystem processes. Second, functional-trait-based  
568 approaches can explicitly address intra-specific variability at the individual level (Albert et al., 2011;

569 Rota et al., 2018) and the findings then scaled up to the community and ecosystem levels (e.g. Lavorel  
570 and Grigulis, 2012).

571       The development of functional-traits-based approaches in trophic ecology is still hampered by  
572 several limitations and drawbacks, but with recent progress these are being overcome e.g. by using  
573 methodological standardization (Moretti et al., 2017). Further, it remains difficult to assess the strength  
574 of species interactions (the direct effect that species have on their mutual demography). Nevertheless,  
575 it is possible to estimate functional food web relationships by distinguishing (1) the life-history traits  
576 which may modulate the strength of an interaction on population growth, from (2) the topological traits  
577 which may modulate the pairwise interactions between the predator and its prey, and from (3) the  
578 consumption traits which may modulate the *per capita* interaction strength (Gravel et al. 2016, see also  
579 Brousseau et al., 2018). Additionally, the correlation between individual traits and how this lack of  
580 independence among traits reflects phylogenetic (evolutionary) constraints remain poorly understood  
581 (Poff et al., 2006). Thus, the validity of linking traits to actual trophic functions continues to be a  
582 source of debate, as the causal link between them is often far from obvious, quite variable, and/or has  
583 yet to be definitively demonstrated.

584

585

586 3.3. Ecological stoichiometry (ES)

587 The consequences of elemental imbalances in ecosystems can be explored using ES approaches, in  
588 which the elemental compositions of heterotrophic organisms (generally expressed as C:N or C:P  
589 ratios) are considered as far less variable than those of primary producers, at least for most similarly  
590 sized taxa (Persson et al., 2010). Based on the law of conservation of matter, ES approaches can  
591 predict how organisms are influenced by the mismatch between consumer demand and the elemental  
592 supply available in resources (Sterner and Elser, 2002). At the ecosystem scale, measuring imbalances  
593 can be used to estimate nutrient transfer efficiency and secondary production (Cebrian et al., 2009). In  
594 the presence of excess elements in their food, consumers make use of several coping strategies such as:  
595 (1) selecting food with elemental ratios close to their requirements and/or (2) eating food of different  
596 qualities but assimilating only those elements that fulfil their requirements while rejecting those  
597 available in excess. In the latter case, mass balance models can estimate the amount of excess elements  
598 released. Experimental measurements of metazoan's nitrogen and phosphorus excretion rates showed  
599 that animals can supply nutrients at rates similar to those of other major nutrient sources and support a  
600 large portion of primary production by nutrient recycling (Vanni, 2002; Vanni et al., 2002). Excreted  
601 elements reduce nutrient limitations and impact ecosystem processes in a predictable manner (Danger  
602 et al., 2009). Accordingly, ES is a powerful conceptual framework for predicting both the  
603 consequences of resource ingestion on consumers' life history traits and the ecosystem consequences  
604 of elemental imbalances. To date, most ES studies have focused on C, N and P; however, the  
605 consideration of other essential elements (e.g. As or Cu, see Karimi and Folt, 2006) might provide a  
606 better understanding of nutrient transfer and the potential limitations for consumer growth or  
607 ecological processes (Welti et al., 2017).

608

609 3.4. Resource quality

610 Parameters other than the C:N:P ratios of resources can influence consumers' growth and physiological  
611 status as well as the efficiency of nutrient and energy transfer into food webs. These include several  
612 essential compounds as crucial drivers of diet selection (Kohl et al., 2015). For example, the lack of  
613 certain polyunsaturated FAs of the  $\omega 3$  series and/or phytosterols limits *Daphnia* growth and/or  
614 reproduction (e.g. Martin-Creuzburg et al., 2009). Micronutrients are another example and include  
615 carotenoids, described in Section 3.1.2. These pigments are important stimulators of immune responses  
616 and show antioxidant properties (e.g. Lucas et al., 2014). Integrative approaches that include large  
617 classes of biochemical compounds (proteins, lipids, glucids) have been proposed to relate resource  
618 composition and organism fitness within a conceptual framework referred to as "nutritional geometry"  
619 (Simpson and Raubenheimer, 1993). Its advantage is that it directly relates consumers' feeding  
620 strategies to their life history traits (e.g. optimal carbohydrate/protein ratios that optimize either  
621 individual growth or reproduction) and thus nutritional geometry allows evolutionary questions to be  
622 explicitly related to trophic ecology. Moreover, a combination of all of these resource-quality-based  
623 approaches has been proposed (Sperfeld et al., 2017) and is a promising methodological strategy for  
624 research in trophic ecology.

625

626

#### 627 4. Testing the implications of trophic interactions (trophic models)

628 While inference methods are used to reconstruct trophic interactions within community (Section 2),  
629 modelling approaches presented in this section allow investigations of the consequences of feeding  
630 interactions on various ecosystem features, ranging from population to ecosystem dynamics. The  
631 assumptions underlying the different models include continuous/discrete, mechanistic/statistical and  
632 individual-/population-based systems. The common goal is to simulate food web functioning from a  
633 set of given conditions, such as network structure, which can in some cases be determined through  
634 inference methods. Modelling approaches can be used to investigate the potential implications of  
635 feeding interactions in terms of community dynamics and/or organic matter/energy transfers (e.g. Rall  
636 et al. 2008) and thus contribute to an improved understanding and management of ecosystems. The use  
637 of models also allows the testing of hypotheses that cannot be tested in natural systems (e.g. Gaucherel  
638 et al., 2017) as well as the formulation of predictions regarding ecosystem responses to management  
639 decisions (e.g. Marzloff et al., 2016). In this section, we present the main modelling approaches  
640 currently used for hypotheses testing in trophic ecology.

641

##### 642 4.1. Ordinary differential equations and qualitative models

643 Ordinary differential equations (ODEs) simulating species dynamics, such as the Lotka-Volterra  
644 equations, can be adapted for food web models. The latter have been central to understanding indirect  
645 interactions in food webs and in studying the consequences of predator-prey interactions on population  
646 dynamics (e.g. Rall et al., 2008). Food web models based on ODEs allow investigations of the  
647 relationship between food web complexity and stability (e.g. McCann and Rooney, 2009). During the  
648 last two decades, models based on ODEs have been expanded to include allometric constraints on  
649 species traits, such as metabolic rates and feeding rates, leading to the so-called bioenergetic or

650 allometric trophic network model (Brose et al., 2017). Ecosim, the time dynamic simulation module  
651 associated with Ecopath, is a well-known application of this approach and it is commonly used to  
652 explore policy options in the ecosystem-based management of fisheries, such as the effects of fishing  
653 on ecosystems and the establishment of marine protected areas (see Walters et al., 1997).

654         Qualitative modelling focuses on the feedback structure of food webs and is used to assess both  
655 stability and ecosystem responses to perturbations (Puccia and Levins, 1985). This method, which is  
656 also based on ODEs, is particularly well suited for trophic ecology studies, as it does not rely on the  
657 quantification of all trophic interactions within a system. Indeed, based only on qualitative assessments  
658 of the interactions among major trophic compartments (e.g. positive, neutral or negative), qualitative  
659 models of food webs can provide a general understanding of ecosystem stability and dynamics based  
660 on network topology (Hulot et al., 2000). For instance, they can be used to predict the overall  
661 responses of a community to long-term disturbances, as the spatial shift of multiple marine species due  
662 to ocean warming (e.g. Marzloff et al. 2016). However, the predictions are most useful for simple  
663 networks (< 15 nodes) as they become ambiguous in more complex systems (e.g. Dambacher et al.,  
664 2003).

#### 665             4.2. Statistical approaches 666

667 Statistical approaches are based on graphical models (see section 2.1), but in the present case, the  
668 graph structure (i.e. the trophic network) is known and is not an output of the model. Trophic  
669 interactions are represented as edges in the graph and are interpreted as statistical interactions between  
670 species by the model. Using the resulting graph, statistical approaches are able to model the probability  
671 of species persistence, explicitly, without a mechanistic model of the extinction dynamics. Unlike  
672 Lotka-Volterra or structural food-web models that are based on material fluxes, statistical approaches  
673 can be built from simple “on-field” observations of individual occurrence. The required experimental

674 set-up is thus simpler than the one for computing the parameters and structure of Lotka-Volterra or  
675 structural models. Another advantage is the possibility to evaluate the resilience of trophic networks  
676 using the probabilities of species persistence, and not only the trophic structure. McDonald-Madden et  
677 al. (2016) adopted this approach to study the conservation management of 6 real food webs (the  
678 Alaskan, Baltic Sea, Lake Vattern, Chesapeake Bay, Arizona Montane forest and Long Island Salt  
679 Marsh food webs) and 40 hypothetical food webs. The resulting predictions allow estimating the effect  
680 of management strategy directly at the scale of food-web and their study provide a computationally  
681 efficient way of identifying the important species to manage in large food webs.

682

#### 683 4.3. Discrete approaches

684 In discrete approaches, networks of trophic interactions are represented by systems made of distinct  
685 and separate components, and characterised by temporally distinct and separated events (e.g. Campbell  
686 et al. 2011). As such, discrete approaches, more than classical trophic models, are particularly  
687 appropriate to consider complex interaction networks (i.e. networks with a high number of direct and  
688 indirect processes), while their behaviour remain under rigorous control. A strong advantage of  
689 discrete models is thus the possibility to model the evolution of trophic networks over time. Studying  
690 ecosystem trajectories constitutes a potential fruitful research avenue for discrete approaches that  
691 could help identifying sustainable paths (the ecosystem is able to persist and evolve) from dead-ends  
692 (the ecosystem disappears or is stuck in a specific state with less interacting species). Gaucherel et al.  
693 (2017) followed such an approach, using Boolean networks, to study the trajectories of a theoretical  
694 termite colony. By removing different numbers and types of nodes, the authors simulated different  
695 disturbances of the colony and examine the effects on the interaction network. By doing so, they  
696 showed that the colony was highly sensitive to the trophic part of the interaction network. In addition,



697 this example illustrates the conceptual shift permitted by discrete approaches toward broader (spatial  
698 and temporal) scales of ecological processes (Wallach et al., 2017).

699

#### 700 4.4. Individual-based models

701 In an individual-based model (IBM), each individual and its associated behaviour are represented,  
702 which allows interactions among individuals to be approximated. IBMs are commonly used in ecology  
703 (Grimm et al. 2005). An important research activity surrounding IBMs consists in understanding how  
704 patterns at the population or community scale emerge from the interactions among individuals (Durrett  
705 and Levin 1994). In trophic ecology, IBMs can be used to reveal the consequences of intraspecific  
706 variations in diet on community trophic structure (Bolnick et al., 2011) or eco-evolutionary dynamics  
707 (Melián et al., 2011). Another application consists in scaling from trait-based interactions between  
708 individuals to the global structure of ecological networks (Jabot and Bascompte, 2012).

709

#### 710 4.5. Dynamic energy and mass budget approaches

711 The dynamic energy and mass budget (DEB) theory describes the organisation of metabolism within  
712 organisms (Sousa et al., 2008) by modelling its quantitative facets (assimilation rate,  
713 growth/maintenance, and maturity/reproduction) based on common rules of allocation and the  
714 processes occurring at the organism level. Standard DEB model consists in a system of three ordinary  
715 differential equations following first order dynamics. Assimilated products first enter a reserve pool  
716 which is then mobilized to fuel two pathways following the kappa rule: a fixed kappa fraction is  
717 allocated to perform growth and maintenance and the remaining fraction (1-kappa) is available for  
718 maturity (i.e. increase of complexity and its maintenance) and reproduction (Van der Meer, 2006). A  
719 set of 12 primary parameters is calibrated from life history traits and univariate data (Lika et al., 2011).

720 Common model inputs are temperature and food availability and outputs are size, shape, full life cycle  
721 dynamics, reproduction rate and composition of the organism (C, H, O, N). Standard DEB models (one  
722 reserve, isometric organism) are commonly used at the level of the individual organism (e.g. Grangeré  
723 et al., 2010) but they can be scaled up to population level (Martin et al, 2012) or to food web and  
724 ecosystem levels including trophic interactions (e.g. Marques et al., 2014). Interesting applications of  
725 DEB models in trophic ecology are related to diet reconstructions (Picoche et al, 2014), the effect of  
726 food stoichiometry on growth (Muller et al, 2001), the effect of prey selection on organism  
727 bioenergetics (Lavaud et al, 2014) and spatio-temporal dynamics of predator-prey interaction  
728 (Grangeré et al., 2010). With some more parameters, they also include the dynamics of body isotope  
729 composition (Pecquerie et al, 2010) and thus offer promising perspectives in understanding  
730 metabolically induced variations in TDFs and in the dynamics of isotope incorporation (Emmery et al.,  
731 2011), in turn allowing better inferences using natural stable isotope ratios.

732

## 733 5. A comprehensive toolbox of methods

734 Trophic ecology is a vibrant field of research supported, as discussed extensively above, by a diverse  
735 and ever-growing array of methodological tools. For ecologists seeking to answer specific trophic  
736 questions, we examine this set of tools (Table 1) based on our own experience and background (Fig.  
737 1). For the empirical methods, our discussion includes the results of a multiple correspondence analysis  
738 (MCA) based on an evaluation of the price, investment, and affinities of each method with key criteria,  
739 such as the observational scale, organisational level, type of ecological question to be tackled and the  
740 type of data produced (Fig. 1A; the procedure is detailed in the Appendix). The first axis of the MCA  
741 was mainly driven by the type of ecological question and the second axis was related to the investment  
742 needed to implement the methods. On the same figure (Fig. 1A), we also displayed the methods'  
743 affinity with organisational level and a rough evaluation of their costs. We completed this analysis with  
744 a subjective classification of the various trophic models (after C. Gaucherel and E. Thebault, pers.  
745 com.). On the resulting Fig. 1B, we focused on the models' affinity with the type of ecological  
746 question and organisational level for the sake of consistency with the classification of empirical  
747 methods. Not included into this classification were inference methods, because they could not be  
748 discriminated by these two axes. The resulting roadmap (Fig. 1A and B) provides an overview of the  
749 affinities of the methods for the different criteria. As an example, from Fig. 1A it can be deduced that  
750 the use of observational methods is more appropriate than stoichiometry if the nature of feeding  
751 interactions is the subject of interest. This visual overview also highlights the gaps (i.e. the absence of  
752 available method to address a specific issue), such as the modelling of fluxes at the integrated scale of  
753 ecosystems (and not only between species, see Fig. 1B). An advantage of the roadmap is that it  
754 facilitates the selection of the best combination of methods with which to investigate (or triangulate) a  
755 specific issue in trophic ecology (see below).

## 756 6. Interconnecting approaches to advance ecological research

757 The abundance of methods currently available to trophic ecologists offers opportunities such as the  
758 triangulation referred to by Munafò and Smith (2018), i.e. the intentional use of multiple approaches  
759 which complementarity offers less-biased, multiple lines of evidence. In other words, combining two  
760 or more methods allow overcoming the shortfalls of each one alone, and initiating interdisciplinary  
761 collaborations that will enrich our knowledge and perspectives. More than combining several methods  
762 at the same integration level (e.g. SIA + FA + Gut contents), we recommend more interconnection of  
763 approaches, for example by creating first a topology using traits, then modelling and using  
764 ingestion/assimilation tracers as validations. Only a few comparative studies of methodologies have  
765 been published (but see Nielsen et al, 2018), in the following we present several examples in which  
766 complementary methods were merged or interconnected successfully and led to novel insights.

767 Combinations of methods have been used to improve empirical descriptions of food webs,  
768 especially as there are recurring issues related to the integration of trophic processes over time. These  
769 can be addressed by complementary approaches. For example, while assimilation tracers such as  
770 isotopes and FAs are regularly combined, the addition of complementary behavioural observations and  
771 ingestion tracers would favour the emergence of comprehensive theories and models describing  
772 tropho-dynamics in food webs (e.g. Woodward et al, 2010, McMeans et al, 2016). Direct field records  
773 (motion-sensitive cameras, Robinson et al, 2018) are also useful additional measurements to obtain  
774 inferred food webs with a much better temporal accuracy. At the individual level, SIAs can be coupled  
775 to DNA-based analyses of gut contents to enable the detection of possible cannibalistic behaviour, as  
776 this is not possible with molecular methods (Traugott et al, 2013), and to overcome the lack of dietary  
777 resolution inherent to stable isotope methods (e.g. Hambäck et al, 2016).

778 Other examples show that interconnecting trait-based concepts with stable isotope methods  
779 sheds light on the interplay between interactions and fluxes. In fact, this has contributed to the  
780 emergence of the concept of isotopic trophic niches (Bowes et al, 2017, and references therein). Also,  
781 placing predator-prey functional traits (e.g. predator biting force vs. prey cuticle toughness) within a  
782 phylogenetic framework (e.g. evolutionary distance between trophic levels) can give rise to models of  
783 predator-prey interactions (Brousseau et al, 2018). Observational data can contribute to inference  
784 methods and models to obtain a characterisation of the ecosystem-level properties emerging from  
785 trophic interactions (e.g. Pacella et al, 2013, de Vries et al, 2013). For example, a Bayesian model  
786 using topology and isotopes was specifically dedicated for the elucidation of trophic strengths in  
787 trophic networks (IsoWeb, Kadoya et al., 2012). Models tested against empirical data can be employed  
788 to reveal the mechanisms operating within food webs and to re-assess the relevant level of complexity  
789 (guilds) at which food webs should be scrutinised in the field (Hulot et al, 2000). Theoretical  
790 modelling and empirical data, connected through a trait-based approach to species and metabolic rates  
791 within allometric trophic networks, can successfully reproduce complex multi-species dynamics in  
792 natural environments (Boit et al. 2012). Among the most recent attempts involve hybrid modelling, in  
793 which models of the overall dynamics of the ecosystem are combined with realistic individual-based  
794 models to link responses at the individual and population levels (Strauss et al, 2017). Also worthy of  
795 mention are stoichiometry-based approaches, which offer a powerful and promising perspective from  
796 which to extend our knowledge about food webs to include biogeochemical consequences and effects  
797 on ecosystem services (Boit et al, 2012, Strauss et al, 2017). A stoichiometric-trait-based approach was  
798 recently described (Meunier et al, 2017), although it requires further development (Welti et al, 2017).  
799 Multivariate DEB models (several substrates, or/and reserves and structures) offer new perspectives to  
800 integrate nutritional geometry and ecological stoichiometry into a metabolic theory through the

801 concept of organisms' homeostasis (Sperfeld et al., 2017). Capturing complex trophic interactions  
802 within or between species could then be modelled by embedding multivariate DEB models in agent-  
803 based modelling approaches (Sperfeld et al., 2017). Finally, DEB models estimate key morphological  
804 traits, such as size, weight, shape, ingestion rates, and range of temperature tolerance. By doing so,  
805 they provide connections to biophysical models (e.g. temperature, wind speed and radiation) and to  
806 nutritional geometry that allow modelling trophic and ecological niches of organisms (Kearney et al.,  
807 2010).

808

809

## 810 7. Including the microbial world in trophic ecology

811 The microbial world has long been treated almost independently from the macroscopic world (Prosser  
812 2007). Conversely, studies of microbial diversity do not explicitly consider the concepts of trophic  
813 ecology. The emergence of molecular approaches has enabled microbial cryptic diversity to be  
814 revealed through genomics, by mapping co-occurrence networks within microbial communities  
815 (Barberán et al, 2012, Peura et al, 2015). However, the implications of the results in terms of trophic  
816 interactions within microbial communities or in relation to the metazoan food web remain poorly  
817 explored (Peura et al, 2015). On the other hand, trophic ecology has not yet explicitly integrated the  
818 role and contribution of complex microbial communities even though the participation of microbes in  
819 aquatic and marine food webs was described more than four decades ago (Pomeroy, 1974), and  
820 heterotrophic protists have been recognized as the major grazers in the oceans (Calbet and Landry,  
821 2004, Sherr and Sherr, 2007). Bacterial and micro-eukaryotic communities are for the most part  
822 included in food web models as broad, unresolved taxonomic or functional groups, with very few  
823 exceptions (Peura et al, 2015, D’Alelio et al, 2016, Weitere et al, 2018). Furthermore, estimates of  
824 trophic chain length fail to consider heterotrophic microbes (Post, 2002), while recent CSIA-based  
825 assessments register heterotrophic microbes in food webs at the same trophic positions as animals  
826 (Steffan et al., 2015).

827 Consequently, our understanding of the “green” and “brown” food webs, including the tropho-  
828 dynamic processes of the detritosphere, emphasised already by Lindeman (1942), remains deficient,  
829 and the potential contribution of the topology of microbial and metazoan networks to ecosystem  
830 stability and resilience barely known (Peura et al, 2015). Several recent methodological developments  
831 may be the first steps in ground-breaking advances in our understanding of micro-macro food webs:  
832 (1) Recent developments in DNA-SIP (Pepe-Ranney et al, 2016) and RNA-SIP (Kramer et al, 2016)

833 allow the tracking and identification of microbial metabolisers of organic substrates up to primary  
834 consumers (see section 1.4.3), thereby improving empirical descriptions of the links within prokaryotes  
835 and protists but also allowing a quantification of the connection to higher trophic levels. (2) By taking  
836 advantage of the surge of metagenomic and metatranscriptomic approaches, microbial ecologists have  
837 been able to link microbial genetic diversity with the functionalities and biogeochemical processes  
838 determined in field samples (see Morales and Holben 2011 and references therein). (3) Information on  
839 taxon-specific feeding selection and the rates measured in experimental settings from microbial and  
840 metazoan organisms can be used as input in Ecopath-like food web models adapted to explicitly  
841 include highly resolved protozoan contributors (D'Alelio et al, 2016). These models can then be  
842 applied to explore the functional consequences of the switches in microbial food web structure on the  
843 overall trophic transfer of matter across the micro-macro continuum (D'Alelio et al, 2016). Ecological  
844 stoichiometry (Welti et al., 2017) or the application of trait-based approaches to microorganisms  
845 (Litchman et al., 2015) may serve as a bridge between the microbial food web and the energetics and  
846 biogeochemistry of the ecosystem, while allometric scaling could connect processes at the food web  
847 scale, thus merging the “green” and “brown” food chains (e.g. Mulder et al., 2013).

848         For trophic ecologists, these elements well evidence the so-far-unrealised opportunity to fully  
849 consider vertical biodiversity based on an explicit representation of the micro- and macroscopic actors  
850 and their roles. With the improvement of the collaboration between microbiologists and trophic  
851 ecologists many long-standing questions will finally be answered and “complexity made simple”  
852 (Pomeroy, 2001).

853

854



## 855 Conclusion

856 In a world of fast and dynamical changes in environmental conditions, there is a crucial need to  
857 understand and anticipate changes in biodiversity in both space and time. Direct effects of changes in  
858 physical factors on species depend on specific tolerance, physiological acclimation, phenotypic  
859 plasticity and evolutionary processes (Angert et al, 2013), likely to create local changes of biodiversity  
860 such as spatial redistribution of species (Marzloff et al, 2016). Yet, evidences are piling that indirect  
861 effects of global changes through alterations in species interactions overweight direct effects (see Holt  
862 and Barfield, 2009, Angert et al, 2013), or that species range shifting can induce new trophic cascades,  
863 inflating the ecological consequences of multiple species redistribution (Marzloff et al, 2016). Such  
864 observations of the crucial role of feeding interactions in the biodiversity response to global changes  
865 place trophic ecology at the core of the issue (see R. Holt contribution to Nature 2020 visions, Norvig  
866 et al, 2010). Recent conceptual and methodological developments in trophic ecology now allow to  
867 explore all the dimensions of such changes, but also to draw cross-scale linkages, at least for some  
868 pilot sites and case studies. Numerical improvements and machine-learning technics offer the  
869 opportunity to test theories and capitalize on these laboriously obtained field- or lab- data by expanding  
870 the time- and space scales of processes and predictions (Faisal et al, 2010; Angert et al, 2013). We  
871 hope this synthesis will convince our colleagues that we have at hand the perfect toolbox to finally deal  
872 with the enormous complexity of trophic implications.

873

## 874 **Acknowledgements**

875 This contribution is a collective effort by the French network for trophic ecology (GRET: Groupe de  
876 Recherche en Ecologie Trophique), supported by CNRS, INEE and INRA as GDR 3716. We are  
877 grateful to the numerous members of the GRET who attended workshops in Toulouse and Nantes,

878 where fruitful discussions led to this review. We address many thanks to Craig Layman for his helpful  
879 comments on a previous draft of our manuscript. None of the authors have any conflict of interest to  
880 declare. MEP proposed this review and NM, NHT and MEP led, organized and completed the writing.  
881 CG provided helpful support for Sections 2 and 4, and ET, AB and MD for Section 6. All authors  
882 contributed critically to the drafts and gave final approval of the manuscript for publication.

883

884 **Literature cited**

- 885 Aderhold, A., Husmeier, D., Lennon, J.J., Beale, C.M. & Smith, V.A. (2012) Hierarchical  
886 Bayesian models in ecology: reconstructing species interaction networks from  
887 nonhomogeneous species abundance data. *Ecological Informatics*, 11, 55–64.
- 888 Albert, C.H., Grassein, F., Schurr, F.M., Vieilledent, G. & Violle, C. (2011) When and how should  
889 intraspecific variability be considered in trait-based plant ecology? *Perspectives in Plant*  
890 *Ecology, Evolution and Systematics*, 13, 217–225.
- 891 Angert, A.L., Ladeau, S.L. & Ostfeld, R.S. (2013) Climate change and species interactions: ways  
892 forward. *Annals of the New York Academy of Sciences*, 1297, 1–7.
- 893 Arts, M.T., Brett, M.T. & Kainz, M.J. (2009) *Lipids in aquatic ecosystems*. Springer, New  
894 York. Baker, R., Buckland, S. & Sheaves, M. (2014) Fish gut content analysis: robust  
895 measures of diet composition. *Fish and Fisheries*, 15, 170–177.
- 896 Baldwin, R.E., Miller, T.W., Brodeur, R.D. & Jacobson, K.C. (2008) Expanding the foraging  
897 history of juvenile Pacific salmon: Combining stomach-content and macroparasite-  
898 community analyses for studying marine diets. *Journal of Fish Biology*, 72, 1268–1294.
- 899 Barberán, A., Bates, S.T., Casamayor, E.O. & Fierer, N. (2012) Using network analysis to explore  
900 co-occurrence patterns in soil microbial communities. *The ISME Journal*, 6, 343–351.
- 901 Bartomeus, I., Gravel, D., Tylianakis, J.M., Aizen, M.A., Dickie, I.A. & Bernard-Verdier, M.  
902 (2016) A common framework for identifying linkage rules across different types of  
903 interactions. *Functional Ecology*, 30, 1894–1903.
- 904 Bastos, R., Corrúa, F., Winemiller, K. & Garcia, A. (2017) Are you what you eat? Effects of  
905 trophic discrimination factors on estimates of food assimilation and trophic position with a  
906 new estimation method. *Ecological Indicators*, 75, 234–241.

907 Bec, A., Perga, M.-E., Koussoroplis, A., Bardoux, G., Desvillettes, C., Bourdier, G. & Mariotti, A.  
908 (2011) Assessing the reliability of fatty acid-specific stable isotope analysis for trophic  
909 studies. *Methods in Ecology and Evolution*, 2, 651–659.

910 Bodé, S., Fancy, R. & Boeckx, P. (2013), Stable isotope probing of amino sugars – a promising  
911 tool to assess microbial interactions in soils. *Rapid Commun. Mass Spectrom.*, 27: 1367–  
912 1379. doi:10.1002/rcm.6586

913 Boecklen, W.J., Yarnes, C.T., Cook, B.A. & James, A.C. (2011) On the use of stable isotopes in  
914 Trophic Ecology. *Annual Review in Ecology and Evolution Systems*, 42, 411–440.

915 Bohan, D.A., Caron-Lormier, G., Muggleton, S., Raybould, A. & Tamaddoni-Nezhad, A. (2011)  
916 Automated discovery of food webs from ecological data using logic-based machine learning.  
917 *PLoS One*, 6, e29028.

918 Boit, A., Martinez, N.D., Williams, R.J. & Gaedke, U. (2012) Mechanistic theory and modelling  
919 of complex food-web dynamics in Lake Constance. *Ecology Letters*, 15, 594–602.

920 Bolnick, D.I., Amarasekare, P., Araujo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf,  
921 V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D.A. (2011) Why intraspecific trait  
922 variation matters in community ecology. *Trends in Ecology & Evolution*, 26, 183–192.

923 Bond, A.L. & Diamond, A.W. (2011) Recent Bayesian stable-isotope mixing models are highly  
924 sensitive to variation in discrimination factors. *Ecological Applications* 21, 1017–1023.

925 Boschker, H.T.S., Nold, S.C., Wellsbury, P. & Bos, D. (1998) Direct linking of microbial  
926 populations to specific biogeochemical processes by <sup>13</sup>C-labelling of biomarkers. *Nature*,  
927 392, 801.

928 Bowes, R.E., Thorp, J.H. & Reuman, D.C. (2017) Multidimensional metrics of niche space for use  
929 with diverse analytical techniques. *Scientific Reports*, 7, srep41599.

930 Bromaghin, J. F. (2017) QFASAR: quantitative fatty acid signature analysis with R. *Methods in*  
931 *Ecology and Evolution*, 8, 1158–1162.

932 Brousseau, P.-M., Gravel, D. & Handa, I.T. (2018). Trait matching and phylogeny as predictors of  
933 predator–prey interactions involving ground beetles. *Functional Ecology*, 32, 192–202.

934 Brose, U., Blanchard, J.L., Eklöf, A., Galiana, N., Hartvig, M., Hirt, M., Kalinkat, G., Nordström,  
935 M.C., O’Gorman, E.J. & Rall, B.C. (2017) Predicting the consequences of species loss using  
936 size-structured biodiversity approaches. *Biological Reviews*, 92, 684–697.

937 Bustamante, P., Caurant, F., Fowler, S.W. & Miramand, P. (1998). Cephalopods as a vector for the  
938 transfer of cadmium to top marine predators in the north-east Atlantic Ocean. *Science of the*  
939 *Total Environment*, 220, 71–80.

940 Bustnes, J.O., Bourgeon, S., Leat, E.H.K., Magnúsdóttir, E., Strøm, H., Hanssen, S.A., Petersen,  
941 A., Olafsdóttir, K., Borgå, K., Gabrielsen, G.W. & Furness, R.W. (2015). Multiple stressors  
942 in a top predator seabird: Potential ecological consequences of environmental contaminants,  
943 population health and breeding conditions. *PLoS One*, 10, e0131769.

944 Calbet, A. & Landry, M.R. (2004) Phytoplankton growth, microzooplankton grazing, and carbon  
945 cycling in marine systems. *Limnology and Oceanography*, 49, 51–57.

946 Campbell, C.E., Yang, S., Albert, R. & Shea, K. (2011) A network model for plant–pollinator  
947 community assembly. *Proceedings of the National Academy of Sciences*, 108, 197–202.

948 Carleton, S.A., Kelly, L., Anderson-Sprecher, R. & Del Rio, C.M. (2008) Should we use one-, or  
949 multi-compartment models to describe <sup>13</sup>C incorporation into animal tissues? *Rapid*  
950 *Communications in Mass Spectrometry*, 22, 3008–3014.

951 Caurant, F. & Amiard-Triquet, C. (1995). Cadmium contamination in pilot whales *Globicephala*  
952 *melas*: source and potential hazard to the species. *Marine Pollution Bulletin*, 30, 207–210.

953 Cebrian, J., Shurin, J.B., Borer, E.T., Cardinale, B.J., Ngai, J.T., Smith, M.D. & Fagan, W.F.  
954 (2009) Producer nutritional quality controls ecosystem trophic structure. *PLoS One*, 4,  
955 e4929.

956 Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P. & Evershed, R.P. (2004) Lipid content and  
957 carbon assimilation in Collembola: implications for the use of compound-specific carbon  
958 isotope analysis in animal dietary studies. *Oecologia*, 139, 325–335.

959 Christensen, V. & Pauly, D. (1992) Ecopath II - a software for balancing steady-state ecosystem  
960 models and calculating network characteristics. *Ecological Modelling*, 61, 169–185.

961 Clinchy, M., Sheriff, M.J. & Zanette, L.Y. (2013) Predator-induced stress and the ecology of fear.  
962 *Functional Ecology*, 27, 56–65.

963 Costantini, D., Rowe, M., Butler, M.W. & McGraw, K.J. (2010) From molecules to living  
964 systems: historical and contemporary issues in oxidative stress and antioxidant ecology:  
965 Issues in oxidative stress and antioxidant ecology. *Functional Ecology*, 24, 950–959.

966 Crotty, F.V., Adl, S.M., Blackshaw, R.P. & Murray, P.J. (2012) Using stable isotopes to  
967 differentiate trophic feeding channels within soil food webs. *Journal of Eukaryotic*  
968 *Microbiology*, 59, 520–526.

969 Cucherousset, J. & Villéger, S. (2015) Quantifying the multiple facets of isotopic diversity: New  
970 metrics for stable isotope ecology. *Ecological Indicators*, 56, 152–160.

971 D’Alelio, D., Libralato, S., Wyatt, T. & D’Alcalà, M.R. (2016) Ecological-network models link  
972 diversity, structure and function in the plankton food-web. *Scientific Reports*, 6, 21806.

973 Dalsgaard, J., John, M.S., Kattner, G., Müller-Navarra, D. & Hagen, W. (2003) Fatty acid trophic  
974 markers in the pelagic marine environment. *Advances in Marine Biology*, 46, 225–340.

975 Dambacher, J.M., Luh, H.-K., Li, H.W. & Rossignol, P.A. (2003) Qualitative stability and  
976 ambiguity in model ecosystems. *The American Naturalist*, 161, 876–888.

977 Danger, M., Lacroix, G., Kâ, S., Corbin, D. & Lazzaro, X. (2009) Food-web structure and  
978 functioning of temperate and tropical lakes: a stoichiometric viewpoint. *International Journal*  
979 *of Limnology*, 45, 11–21.

980 De Vries, F.T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M.A., Bjørnlund, L., Bracht  
981 Jørgensen, H., Vincent Brady, M., Christensen, S., de Ruiter, P.C., d’Hertefeldt, T., Frouz,  
982 J., Hedlund, K., Hemerik, L., Hol, G.W.H., Hotes, S., Mortimer, S.R., Setälä, H., Sgardelis,  
983 S.P., Uteseny, K., van der Putten, W.H., Wolters, V. & Bardgett, R.D. (2013) Soil food web  
984 properties explain ecosystem services across European land use systems. *Proceedings of the*  
985 *National Academy of Sciences*, 110, 14296–14301.

986 Dean, T. & Kanazawa, K. (1989) A model for reasoning about persistence and causation.  
987 *Computational Intelligence*, 5, 142–150.

988 Del Rio, C.M., Wolf, N., Carleton, S.A. & Gannes, L.Z. (2009) Isotopic ecology ten years after a  
989 call for more laboratory experiments. *Biological Reviews*, 84, 91–111.

990 Derycke, S., De Meester, N., Rigaux, A., Creer, S., Bik, H., Thomas, W.K. & Moens, T. (2016)  
991 Coexisting cryptic species of the *Litoditis marina* complex (Nematoda) show differential  
992 resource use and have distinct microbiomes with high intraspecific variability. *Molecular*  
993 *Ecology*, 25, 2093–2110.

994 Deshpande A.D., Dickhut R.M., Dockum B.W., Brill R.W. & Farrington, C. (2016)  
995 Polychlorinated biphenyls and organochlorine pesticides as intrinsic tracer tags of foraging  
996 grounds of bluefin tuna in the northwest Atlantic Ocean. *Marine Pollution Bulletin*, 105,  
997 265–276.

- 998 Desvillettes, C., Bourdier, G., Amblard, C. & Barth, B. (1997) Use of fatty acids for the assessment  
999 of zooplankton grazing on bacteria, protozoans and microalgae. *Freshwater Biology*, 38,  
1000 629–637.
- 1001 Duffy, D. C. & Jackson, S. (1986) Diet studies of seabirds: a review of methods. *Colonial*  
1002 *Waterbirds*, 9, 1–17.
- 1003 Dungait, J.A.J., Bol, R., Bull, I.D. & Evershed, R.P. (2009) Tracking the fate of dung-derived  
1004 carbohydrates in a temperate grassland soil using compound-specific stable isotope analysis.  
1005 *Organic Geochemistry*, 40, 1210–1218.
- 1006 Dupuy, C., Rossignol, L., Geslin, E. & Pascal, P.-Y. (2010) Predation of mudflat meio-  
1007 macrofaunal metazoans by a calcareous foraminifer, *Ammonia tepida* (Cushman, 1926).  
1008 *Journal of Foraminiferal Research*, 40, 305–312.
- 1009 Durrett, R. & Levin, S. (1994) The importance of being discrete (and spatial). *Theoretical*  
1010 *Population Biology*, 46, 363–394.
- 1011 Ebeling, A., Rzanny, M., Lange, M., Eisenhauer, N., Hertzog, L.R., Meyer, S.T. & Weisser, W.W.  
1012 (2018) Plant diversity induces shifts in the functional structure and diversity across trophic  
1013 levels. *Oikos*, 127, 208–219.
- 1014 Eitzinger, B., Micic, A., Körner, M., Traugott, M. & Scheu, S. (2013) Unveiling soil food web  
1015 links: New PCR assays for detection of prey DNA in the gut of soil arthropod predators. *Soil*  
1016 *Biology and Biochemistry*, 57, 943–945
- 1017 Elser, J.J., Dobberfuhl, D.R., MacKay, N.A. & Schampel, J.H. (1996) Organism size, life history,  
1018 and N:P stoichiometry. *BioScience*, 46, 674–684.
- 1019 Emmery, A., Lefebvre, S., Alunno-Bruscia, M. & Kooijman, S.A.L.M. (2011) Understanding the  
1020 dynamics of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in soft tissues of the bivalve *Crassostrea gigas* facing



1021 environmental fluctuations in the context of Dynamic Energy Budgets (DEB). *Journal of Sea*  
1022 *Research*, 66, 361–371.

1023 Faisal, A., Dondelinger, F. , Husmeier, D. & Beale, C.M. (2010) Inferring species interaction  
1024 networks from species abundance data: A comparative evaluation of various statistical and  
1025 machine learning methods. *Ecological Informatics*, 5, 451–464.

1026 Fueser, H., Majdi, N., Haegerbaeumer, A., Pilger, C., Hachmeister, H., Greife, P., Huser, T. &  
1027 Traunspurger, W. (2018) Analyzing life-history traits and lipid storage using CARS  
1028 microscopy for assessing effects of copper on the fitness of *Caenorhabditis elegans*.  
1029 *Ecotoxicology and Environmental Safety*, 156, 255–262.

1030 Friedman, J., Hastie, T. & Tibshirani, R. (2008) Sparse inverse covariance estimation with the  
1031 graphical lasso. *Biostatistics*, 9, 432–441.

1032 Fry, B. (2006) *Stable isotope ecology*, Springer, Berlin.

1033 Gannes, L.Z., O’Brien, D.M. & Del Rio, C.M. (1997) Stable isotopes in animal ecology:  
1034 assumptions, caveats, and a call for more laboratory experiments. *Ecology*, 78, 1271–1276.

1035 Gannes, L.Z., Del Rio, C.M. & Koch, P. (1998) Natural abundance variations in stable isotopes  
1036 and their potential uses in animal physiological ecology. *Comparative Biochemistry and*  
1037 *Physiology A*, 119, 725–737.

1038 Garvey, J.E. & Whiles, M.R. (2017) *Trophic Ecology*, CRC Press, Boca Raton, FL

1039 Gaucherel, C., Théro, H., Puiseux, A. & Bonhomme, V. (2017) Understand ecosystem regime  
1040 shifts by modelling ecosystem development using Boolean networks. *Ecological*  
1041 *Complexity*, 31, 104–114.

- 1042 Gladyshev, M.I., Makhutova, O.N., Kravchuk, E.S., Anishchenko, O.V. & Sushchik N.N. (2016)  
1043 Stable isotope fractionation of fatty acids of *Daphnia* fed laboratory cultures of microalgae/  
1044 *Limnologica*, 56, 23–29.
- 1045 González-Varo, J.P. & Traveset, A. (2016) The labile limits of forbidden interactions. *Trends in*  
1046 *Ecology & Evolution*, 31, 700–710.
- 1047 Grangeré, K., Lefebvre, S., Bacher, C., Cugier, P. & Ménesguen, A. (2010) Modelling the spatial  
1048 heterogeneity of ecological processes in an intertidal estuarine bay: dynamic interactions  
1049 between bivalves and phytoplankton. *Marine Ecology Progress Series*, 415, 141–158.
- 1050 Gravel, D., Albouy, C. & Thuiller, W. (2016) The meaning of functional trait composition of food  
1051 webs for ecosystem functioning. *Philosophical Transactions of the Royal Society B*, 371,  
1052 20150268.
- 1053 Green, A.J. (2001) Mass/length residuals: Measures of body condition or generators of spurious  
1054 results? *Ecology*, 82, 1473–1483.
- 1055 Grimm, V., Revilla, E., Berger, U., Jeltsch, F., Mooij, W.M., Railsback, S.F., Thulke, H.-H.,  
1056 Weiner, J., Wiegand, T. & DeAngelis, D.L. (2005) Pattern-oriented modeling of agent-based  
1057 complex systems: lessons from ecology. *Science*, 310(5750), 987-991.
- 1058 Hall, R.O. & Meyer, J.L. (1998) The trophic significance of bacteria in a detritus-based stream  
1059 food web. *Ecology*, 79, 1995–2012.
- 1060 Hambäck, P.A., Weingartner, E., Dalén, L., Wirta, H. & Roslin, T. (2016) Spatial subsidies in  
1061 spider diets vary with shoreline structure: Complementary evidence from molecular diet  
1062 analysis and stable isotopes. *Ecology and Evolution*, 6, 8431–8439.

- 1063 Happel, A., Stratton, L., Kolb C., Hays, C., Rinchard, J. & Czesny S. (2016) Evaluating  
1064 quantitative fatty acid signature analysis (QFASA) in fish using controlled feeding  
1065 experiments. *Canadian Journal of Fisheries and Aquatic Sciences*, 73, 1222–1229.
- 1066 Harrison, X.A., Blount, J.D., Inger, R., Norris, D.R. & Bearhop, S. (2011) Carry-over effects as  
1067 drivers of fitness differences in animals: Carry-over effects in animal populations. *Journal of*  
1068 *Animal Ecology*, 80, 4–18.
- 1069 Hebert, C.E., Weseloh, D.V.C., Gauthier, L.T., Arts, M.T. & Letcher, R.J. (2009) Biochemical  
1070 tracers reveal intra-specific differences in the food webs utilized by individual seabirds.  
1071 *Oecologia*, 160, 15–23.
- 1072 Heidemann, K., Ruess, L., Scheu, S. & Maraun, M. (2014) Nematode consumption by mite  
1073 communities varies in different forest microhabitats as indicated by molecular gut content  
1074 analysis. *Experimental and Applied Acarology*, 64, 49–60.
- 1075 Ho, T.W., Hwang, J.-S., Cheung, M.K., Kwan, H.S. & Wong, C.K. (2017) DNA-based study of  
1076 the diet of the marine calanoid copepod *Calanus sinicus*. *Journal of Experimental Marine*  
1077 *Biology and Ecology*, 494, 1–9.
- 1078 Højsgaard, S., Edwards, D. & Lauritzen, S. (2012) Gaussian Graphical Models. *Graphical Models*  
1079 *with R. Use R!* Springer, Berlin.
- 1080 Holmstad, P.R., Holstad, Ø., Karbøl, G., Revhaug, J.O., Schei, E., Vandvik, V. & Skorping, A.  
1081 (2004) Parasite tags in ecological studies of terrestrial hosts: a study on ptarmigan  
1082 (*Lagopus* spp.) dispersal. *Ornis Fennica*, 81, 128–136.
- 1083 Hulot, F.D., Lacroix, G., Lescher-Moutoué, F. & Loreau, M. (2000) Functional diversity governs  
1084 ecosystem response to nutrient enrichment. *Nature*, 405, 340–344.

1085 Holt, R.D. & Barfield, M. (2009) Trophic interactions and range limits: the diverse roles of  
1086 predation. *Proceedings of the Royal Society B: Biological Sciences*, 276, 1435–1442.

1087 Hyndes, G., Hanson, C. & Vanderklift, M. (2013) The magnitude of spatial and temporal variation  
1088 in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  differs between taxonomic groups: Implications for food web studies.  
1089 *Estuarine, Coastal and Shelf Science*, 119, 176–187.

1090 Ibanez, S., Manneville, O., Miquel, C., Taberlet, P., Valentini, A., Aubert, S., Coissac, E., Colace,  
1091 M.-P., Duparc, Q. & Lavorel, S. (2013) Plant functional traits reveal the relative contribution  
1092 of habitat and food preferences to the diet of grasshoppers. *Oecologia*, 173, 1459–1470.

1093 Iverson, S.J., Field, C., Bowen, W.D. & Blanchard, W. (2004) Quantitative fatty acid signature  
1094 analysis: a new method of estimating predator diets. *Ecological Monographs*, 74, 211–235.

1095 Jabot, F. & Bascompte, J. (2012) Biotrophic interactions shape biodiversity in space. *Proceedings of*  
1096 *the National Academy of Sciences*, 109, 4521–4526.

1097 Jabot, F., Faure, T. & Dumoulin, N. (2013) EasyABC: performing efficient approximate Bayesian  
1098 computation sampling schemes using R. *Methods in Ecology and Evolution*, 4, 684–687.

1099 Jackson, A.L., Parnell, A.C., Inger R. & Bearhop, S. (2011) Comparing isotopic niche widths  
1100 among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *Journal of*  
1101 *Animal Ecology*, 80, 595–602.

1102 Jakob, E.M., Marshall, S.D. & Uetz, G.W. (1996) Estimating fitness: A comparison of body  
1103 condition indices. *Oikos*, 77, 61–67.

1104 Jensen, F.V. & Nielsen, T.D. (2007) *Bayesian networks and decision graphs*, Springer, Berlin.

1105 Jordano, P. (2016) Sampling networks of ecological interactions. *Functional Ecology*, 30, 1883–  
1106 1893.

- 1107 Kadoya, T., Osada, Y. & Takimoto, G. (2012) IsoWeb: A Bayesian isotope mixing model for diet  
1108 analysis of the whole food web. *PLoS One*, 7, e41057.
- 1109 Karimi, R. & Folt, C.L. (2006) Beyond macronutrients: element variability and multielement  
1110 stoichiometry in freshwater invertebrates. *Ecology Letters*, 9, 1273–1283.
- 1111 Kearney, M., Simpson, S.J., Raubenheimer, D. & Helmuth, B. (2010) Modelling the ecological  
1112 niche from functional traits. *Philosophical Transactions of the Royal Society B*, 365, 3469–  
1113 3483.
- 1114 Kelly, B.C., Ikonou, M.G., Blair, J.D., Morin, A.E. & Gobas, F.A.P.C. (2007) Food web-  
1115 specific biomagnification of persistent organic pollutants. *Science*, 317, 236–239.
- 1116 King, R.A., Read, D.S., Traugott, M. & Symondson, W.O.C. (2008) Molecular analysis of  
1117 predation: a review of best practice for DNA-based approaches. *Molecular Ecology*, 17,  
1118 947–963.
- 1119 Klein-Breteler, W.C.M., Schogt, N., Baas, M., Schouten, S. & Kraay, G.W. (1999) Trophic  
1120 upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids.  
1121 *Marine Biology*, 135, 191–198.
- 1122 Kohl, K.D., Coogan, S.C.P. & Raubenheimer, D. (2015) Do wild carnivores forage for prey or for  
1123 nutrients? *BioEssays*, 37, 701–709.
- 1124 Koussoroplis, A.-M., Bec, A., Perga, M.-E., Koutrakis, E., Desvillettes, C. & Bourdier, G. (2010)  
1125 Nutritional importance of minor dietary sources for leaping grey mullet *Liza saliens*  
1126 (*Mugilidae*) during settlement: insights from fatty acid  $\delta^{13}C$  analysis. *Marine Ecology*  
1127 *Progress Series*, 404, 207–217.
- 1128 Kramer, S., Dibbern, D., Moll, J., Huenninghaus, M., Koller, R., Krueger, D., Marhan, S., Urich,  
1129 T., Wubet, T., Bonkowski, M., Buscot, F., Lueders, T. & Kandeler, E. (2016) Resource

1130 partitioning between bacteria, fungi, and protists in the detritusphere of an Agricultural Soil  
1131 (2016) *Frontiers in Microbiology*, 7, 1524.

1132 Krahn, M.M., Herman, D.P., Matkin, C.O., Durban, J.W., Barrett-Lennard, L., Burrows, D.G.,  
1133 Dahlheim, M.E., Black, N., LeDuc, R.G. & Wade, P.R. (2007) Use of chemical tracers in  
1134 assessing the diet and foraging regions of eastern North Pacific killer whales. *Marine*  
1135 *Environmental Research*, 63, 91–114.

1136 Kusmer, K.D. (1990) Taphonomy of owl pellet deposition. *Journal of Paleontology*, 64, 629–637.

1137 Labocha, M.K. & Hayes, J.P. (2012) Morphometric indices of body condition in birds: a review.  
1138 *Journal of Ornithology*, 153, 1–22.

1139 Labocha, M.K., Schutz, H. & Hayes, J.P. (2014) Which body condition index is best? *Oikos*, 123,  
1140 111–119.

1141 Lahaye, V., Bustamante, P., Spitz, J., Dabin, W., Das, K., Pierce, G.J. & Caurant, F. (2005) Long-  
1142 term dietary segregation of short-beaked common dolphins (*Delphinus delphis*) in the Bay of  
1143 Biscay determined using cadmium as an ecological tracer. *Marine Ecology Progress Series*,  
1144 305, 275–285.

1145 Larsson, P., Woin, P. & Knulst, J. (1990) Differences in uptake of persistent pollutants for  
1146 predators feeding in aquatic and terrestrial habitats. *Holarctic Ecology*, 13, 149–155.

1147 Lavaud, R., Flye-Sainte-Marie, J., Jean, F., Emmery, A., Strand, O. & Kooijman, S.A.L.M. (2014)  
1148 Feeding and energetics of the great scallop, *Pecten maximus*, through a DEB model. *Journal*  
1149 *of Sea Research*, 94, 5–18.

1150 Lavorel, S. & Grigulis, K. (2012) How fundamental plant functional trait relationships scale-up to  
1151 trade-offs and synergies in ecosystem services. *Journal of Ecology*, 100, 128–140.

1152 Layman, C.A., Giery, S.T., Buhler, S., Rossi, R., Penland, T., Henson, M.N., Bogdanoff, A.K.,  
1153 Cove, M.V., Irizarry, A.D., Schalk, C.M. & Archer, S.K. (2015) A primer on the history of  
1154 food web ecology: Fundamental contributions of fourteen researchers. *Food Webs*, 4, 14–24.

1155 Legler, J.M. & Sullivan, L.J. (1979) The application of stomach-flushing to lizards and anurans.  
1156 *Herpetologica*, 35, 107–110.

1157 Levine, J.M. (2016) Ecology: A trail map for trait-based studies. *Nature*, 529, 163–164.

1158 Lika, K., Kearney, M.R., Freitas, V., Van Der Veer, H.W., Van Der Meer, J., Wijsman, J.W.M.,  
1159 Pecquerie, L. & Kooikman, S.A.L.M. (2011) The "covariation method" for estimating the  
1160 parameters of the standard Dynamic Energy Budget model I: Philosophy and approach.  
1161 *Journal of Sea Research* 66, 270–277.

1162 Linchant, J., Lisein, J., Semeki, J., Lejeune, P. & Vermeulen, C. (2015) Are unmanned aircraft  
1163 systems (UASs) the future of wildlife monitoring? A review of accomplishments and  
1164 challenges. *Mammal Review*, 45, 239–252.

1165 Lindeman, R.L. (1942) The trophic-dynamic aspect of ecology. *Ecology*, 23, 399–417.

1166 Litchman, E., Edwards, K.F. & Klausmeier C.A. (2015) Microbial resource utilization traits and  
1167 trade-offs: implications for community structure, functioning, and biogeochemical impacts at  
1168 present and in the future. *Frontiers in Microbiology*. 6, 254.

1169 Lotka, A.J. (1925) *Elements of physical biology*. 495pp. Waverly Press, Baltimore, MD.

1170 Lucas, A., Morales, J. & Velando, A. (2014) Differential effects of specific carotenoids on  
1171 oxidative damage and immune response of gull chicks. *Journal of Experimental Biology*,  
1172 217, 1253–1262.

1173 Lueders, T., Wagner, B., Claus, P. & Friedrich, M.W. (2003) Stable isotope probing of rRNA and  
1174 DNA reveals a dynamic methylotroph community and trophic interactions with fungi and  
1175 protozoa in oxic rice field soil. *Environmental Microbiology*, 6, 60–72.

1176 MacKenzie, K. (2002) Parasites as biological tags in population studies of marine organisms: an  
1177 update. *Parasitology*, 124, 153–163.

1178 MacKenzie, K. & Abaunza, P. (1998) Parasites as biological tags for stock discrimination of  
1179 marine fish: A guide to procedures and methods. *Fisheries research*, 38, 45–56.

1180 Majdi, N., Tackx, M., Traunspurger, W. & Buffan-Dubau, E. (2012) Feeding of biofilm-dwelling  
1181 nematodes examined using HPLC-analysis of gut pigment contents. *Hydrobiologia*, 680,  
1182 219–232.

1183 Marcogliese, D.J. (2004) Parasites: small players with crucial roles in the ecological theater.  
1184 *Ecohealth*, 1, 151–164.

1185 Marcogliese, D.J. & Cone, D.K. (1997) Parasite communities as indicators of ecosystem stress.  
1186 *Parasitologia*, 39, 227–232.

1187 Marques, G.M., Mateus, M. & Domingos, T. (2014) Can we reach consensus between marine  
1188 ecological models and DEB theory? A look at primary producers. *Journal of Sea Research*,  
1189 94, 92–104.

1190 Martin, B.T., Zimmer, E. I., Grimm, V. & Jager, T. (2012) Dynamic Energy Budget theory meets  
1191 individual-based modelling: a generic and accessible implementation. *Methods in Ecology  
1192 and Evolution*, 3, 445–449.

1193 Martin-Creuzburg, D., Sperfeld, E. & Wacker, A. (2009) Colimitation of a freshwater herbivore by  
1194 sterols and polyunsaturated fatty acids. *Philosophical Transactions of the Royal Society B*,  
1195 276, 1805–1814.



1196 Marzloff, M.P., Melbourne-Thomas, J., Hamon, K.G., Hoshino, E., Jennings, S., Putten, I. E. &  
1197 Pecl, G.T. (2016) Modelling marine community responses to climate-driven species  
1198 redistribution to guide monitoring and adaptive ecosystem-based management. *Global*  
1199 *Change Biology*, 22, 2462–2474.

1200 Maxfield, P.J., Dildar, N., Hornibrook, E.R.C., Stott, A.W. & Evershed, R.P. (2012) Stable isotope  
1201 switching (SIS): a new stable isotope probing (SIP) approach to determine carbon flow in the  
1202 soil food web and dynamics in organic matter pools. *Rapid Communications in Mass*  
1203 *Spectrometry*, 26, 997–1004.

1204 McCann, K.S. & Rooney, N. (2009) The more food webs change, the more they stay the same.  
1205 *Philosophical Transactions of the Royal Society B*, 364, 1789–1801.

1206 McClelland, J.W. & Montoya, J.P. (2002) Trophic relationships and the nitrogen isotopic  
1207 composition of amino acids in plankton. *Ecology*, 83, 2173–2180.

1208 McDonald-Madden, E., Sabbadin, R., Game, E.T., Baxter, P.W.J., Chadès, I. & Possingham, H.P.  
1209 (2016) Using food-web theory to conserve ecosystems. *Nature Communications*, 7, 10245.

1210 McGuire, L.P. & Guglielmo, C.G. (2010) Quantitative magnetic resonance: a rapid, noninvasive  
1211 body composition analysis technique for live and salvaged bats. *Journal of Mammalogy*, 91,  
1212 1375–1380.

1213 McMeans, B.C., McCann, K.S., Tunney, T.D., Fisk, A.T., Muir, A.M., Lester, N., Shuter, B. &  
1214 Rooney, N. (2016) The adaptive capacity of lake food webs: from individuals to ecosystems.  
1215 *Ecological Monographs*, 86, 4–19.

1216 McWilliams, S.R. (2011) *Ecology of Vertebrate Nutrition*. In: eLS. John Wiley & Sons Ltd,  
1217 Chichester. <http://www.els.net>.

1218 Melián, C.J., Křivan, V., Altermatt, F., Starý, P., Pellissier, L. & De Laender, F. (2015) Dispersal  
1219 dynamics in food webs. *The American Naturalist*, 185, 157–168.

1220 Melián, C.J., Vilas, C., Baldo, F., Gonzalez-Ortegon, E., Drake, P. & Williams, R.J. (2011) Eco-  
1221 evolutionary dynamics of individual-based food webs. *Advances in Ecological Research*, 45,  
1222 225–268.

1223 Meunier, C.L., Boersma, M., El-Sabaawi, R., Halvorson, H.M., Herstoff, E.M., Van de Waal,  
1224 D.B., Vogt, R.J. & Litchman, E. (2017) From elements to function: Toward unifying  
1225 ecological stoichiometry and trait-based ecology. *Frontiers in Environmental Science*, 5, 18.

1226 Mialet, B., Majdi, N., Tackx, M., Azémar, F. & Buffan-Dubau, E. (2013) Selective feeding of  
1227 Bdelloid rotifers in river biofilms. *PLoS One*, 8, e75352.

1228 Middelburg, J.J., Barranguet, C., Boschker, H.T.S., Herman, P.M.J., Moens, T. & Heip, C. H.R.  
1229 (2000) The fate of intertidal microphytobenthos carbon: An in situ <sup>13</sup>C-labeling study.  
1230 *Limnology and Oceanography*, 45, 1224–1234.

1231 Middelburg, J.J. (2014) Stable isotopes dissect aquatic food webs from the top to the bottom.  
1232 *Biogeosciences*, 11, 2357–2371.

1233 Moll, R.J., Millspaugh, J.J., Beringer, J., Sartwell, J. & He, Z. (2007) A new 'view' of ecology and  
1234 conservation through animal-borne video systems. *Trends in Ecology & Evolution*, 22, 660–  
1235 668.

1236 Molnar, P.K., Klanjscek, T., Derocher, A.E., Obbard, M.E. & Lewis, M.A. (2009) A body  
1237 composition model to estimate mammalian energy stores and metabolic rates from body  
1238 mass and body length, with application to polar bears. *Journal of Experimental Biology*, 212,  
1239 2313–2323.

1240 Morales, S.E. & Holben, W.E. (2011) Linking bacterial identities and ecosystem processes: can  
1241 'omic' analyses be more than the sum of their parts? *FEMS Microbiology Ecology*, 72, 2–16

1242 Moretti, M., Dias, A.T.C., Bello, F., Altermatt, F., Chown, S.L., Azcarate, F.M., Bell, J.R.,  
1243 Fournier, B., Hedde, M., Hortal, J., Ibanez, S., Öckinger, E., Sousa, J. P., Ellers, J. & Berg,  
1244 M.P. (2017) Handbook of protocols for standardized measurement of terrestrial invertebrate  
1245 functional traits. *Functional Ecology*, 31, 558–567.

1246 Muggleton, S. (1991) Inductive logic programming. *New Generation Computing*, 8, 295–318.

1247 Muggleton, S.H., Lin, D., Pahlavi, N. & Tamaddoni-Nezhad, A. (2014) Meta-interpretive learning:  
1248 application to grammatical inference. *Machine Learning*, 94, 25–49.

1249 Mulder, C., Ahrestani, F.S., Bahn, M., Bohan, D.A., Bonkowski, M., Griffiths, B.S., Guicharnaud,  
1250 R.A., Kattge, J., Krogh, P.H., Lavorel, S., Lewis, O.T., Mancinelli, G., Naeem, S., Peñuelas,  
1251 J., Poorter, H., Reich, P.B., Rossi, L., Rusch, G.M., Sardans, J. & Wright, I. J. (2013)  
1252 Connecting the green and brown worlds : allometric and stoichiometric predictability of  
1253 above- and below-ground networks. *Advances in Ecological Research*, 49, 69–175.

1254 Muller, E.B., Nisbet, R.M., Kooijman, S., Elser, J.J. & McCauley, E. (2001) Stoichiometric food  
1255 quality and herbivore dynamics. *Ecology Letters*, 4, 519–529.

1256 Munafò, M.R. & Smith, G.D. (2018) Robust research needs many lines of evidence. *Nature*, 553,  
1257 399–401.

1258 Neufeld, J.D., Dumont, M.G., Vohra, J. & Murrell, C.J. (2007) Methodological considerations for  
1259 the use of stable isotope probing in microbial ecology. *Microbial Ecology*, 53, 435–442.

1260 Nielsen, J.M., Clare, E.L., Hayden, B., Brett, M.T. & Kratina, P. (2018) Diet tracing in ecology :  
1261 method comparison and selection. *Methods in Ecology and Evolution*, 9, 278–291.

1262 Norvig, P., Relman, D.A., Goldstein, D.B., Kammen, D.M., Weinberger, D.R., Aiello LC &  
1263 Nicholson, J.K. (2010) 2020 visions. *Nature*, 463, 26–32.

1264 Pace, M.L., Carpenter, S.R., Cole, J.J., Coloso, J.J., Kitchell, J.F., Hodgson, J.R., Middelburg, J.J.,  
1265 Preston, N.D., Solomon, C.T. & Weidel, B.C. (2007) Does terrestrial organic carbon  
1266 subsidize the planktonic food web in a clear-water lake? *Limnology and Oceanography*, 52,  
1267 2177–2189.

1268 Pacella, S.R., Lebreton, B., Richard, P., Phillips, D., DeWitt, T.H. & Niquil, N. (2013)  
1269 Incorporation of diet information derived from Bayesian stable isotope mixing models into  
1270 mass-balanced marine ecosystem models: A case study from the Marennes-Oléron Estuary,  
1271 France. *Ecological Modelling*, 267, 127–137.

1272 Parrish, C.C. (2013) Lipids in marine ecosystems. *ISRN Oceanography*, 2013, 604045.

1273 Parnell, A., Phillips, D., Bearhop, S., Semmens, B., Ward, E., Moore, J., Jackson, A., Grey, J.,  
1274 Kelly, D. & Inger, R. (2013) Bayesian stable isotope mixing models. *Environmetrics*, 24,  
1275 387–399.

1276 Pecquerie, L., Nisbet, R.M., Fablet, R., Lorrain, A. & Kooijman, S.A.L.M. (2010) The impact of  
1277 metabolism on stable isotope dynamics: a theoretical framework. *Philosophical Transactions*  
1278 *of the Royal Society B*, 365, 3455–3468.

1279 Peig, J. & Green, A.J. (2009) New perspectives for estimating body condition from mass/length  
1280 data: the scaled mass index as an alternative method. *Oikos*, 118, 1883–1891.

1281 Pepe-Ranney, C., Campbell, A.N., Koechli, C.N., Berthrong, S. & Buckley, D.H. (2016)  
1282 Unearthing the ecology of soil microorganisms using a high resolution DNA-SIP approach  
1283 to explore cellulose and xylose metabolism in soil. *Frontiers in Microbiology*, 7, 703.

1284 Pepin, P., Parrish, C.C. & Head, E.J.H. (2011) Late autumn condition of *Calanus finmarchicus* in  
1285 the northwestern Atlantic: evidence of size-dependent differential feeding. *Marine Ecology*  
1286 *Progress Series*, 423, 155–166.

1287 Perga, M.E. & Gerdeaux, D. (2005) ‘Are fish what they eat’ all year round? *Oecologia*, 144, 598–  
1288 606.

1289 Perga, M.E. & Grey, J. (2010) Laboratory measures of isotope discrimination factors: comments  
1290 on Caut, Angulo & Courchamp (2008, 2009). *Journal of Applied Ecology*, 47, 942–947

1291 Persson, J., Fink, P., Goto, A., Hood, J.M., Jonas, J. & Kato, S. (2010) To be or not to be what you  
1292 eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos*,  
1293 119, 741–751.

1294 Peura, S., Bertilsson, S., Jones, R.I. & Eiler, A. (2015) Resistant microbial cooccurrence patterns  
1295 inferred by network topology. *Applied and Environmental Microbiology*, 81, 2090–2097.

1296 Philips, D.L. (2012) Converting isotope values to diet composition: The use of mixing models.  
1297 *Journal of Mammalogy*, 93, 342–352.

1298 Picoche, C., Legendr, R., Flye-Sainte-Marie, J., Françoise, S., Maheux, F., Simon, B. & Gangnery,  
1299 A. (2014) Towards the determination of *mytilus edulis* food preferences using the Dynamic  
1300 Energy Budget (DEB) Theory. *Plos One* 9, e109796.

1301 Pierce, G. J. & Boyle, P. R. (1991) A review of methods for diet analysis in piscivorous marine  
1302 mammals. *Oceanography and Marine Biology*, 29, 409–486.

1303 Pitt, J.A., Drouillard, K.G. & Paterson, G. (2017) Polychlorinated biphenyl bioaccumulation  
1304 patterns among Lake Erie lower trophic level consumers reflect species ecologies. *Bulletin*  
1305 *of Environmental Contamination and Toxicology*, 98, 65–70.

- 1306 Poff, N.L.R., Olden, J.D., Vieira, N.K.M., Finn, D.S., Simmons, M.P. & Kondratieff, B.C. (2006)  
1307 Functional trait niches of North American lotic insects: traits-based ecological applications  
1308 in light of phylogenetic relationships. *Journal of the North American Benthological Society*,  
1309 25, 730–755.
- 1310 Pomeroy, L.R. (1974) The ocean's food web, a changing paradigm. *BioScience*, 24, 499–504.
- 1311 Pomeroy, L.R. (2001). Caught in the food web: complexity made simple? *Scientia Marina*, 65, 31–  
1312 40.
- 1313 Post, D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and  
1314 assumptions. *Ecology*, 83, 703–718.
- 1315 Post, D.M. & Palkovacs E.P. (2009) Eco-evolutionary feedbacks in community and ecosystem  
1316 ecology: interactions between the ecological theatre and the evolutionary play. *Philosophical*  
1317 *Transactions of the Royal Society B*, 364, 1629–1640.
- 1318 Poulin, R. (2010) Parasite manipulation of host behavior: an update and frequently asked  
1319 questions. *Advances in the Study of Behavior*, 41, 151–186.
- 1320 Praca, E., Laran, S., Lepoint, G., Thomé, J.P., Quetglas, A., Belcari, P., Sartor, P., Dhermain, F.,  
1321 Ody, D., Tapie, N., Budzinski, H. & Das, K. (2011) Toothed whales in the northwestern  
1322 Mediterranean: Insight into their feeding ecology using chemical tracers. *Marine Pollution*  
1323 *Bulletin*, 62, 1058–1065.
- 1324 Prasetya, F.S., Comeau, L.A., Gastineau, R., Decottignies, P., Cognie, B., Morançais, M.,  
1325 Turcotte, F., Mouget, J.-L. & Tremblay, R. (2017) Effect of marennine produced by the blue  
1326 diatom *Haslea ostrearia* on behavioral, physiological and biochemical traits of juvenile  
1327 *Mytilus edulis* and *Crassostrea virginica*. *Aquaculture*, 467, 138–148.

1328 Prosser, J.I., Bohannan, B.J.M., Curtis, T.P., Ellis, R.J., Firestone, M.K., Freckleton, R.P., Green,  
1329 J.L., Green, L.E., Killham, K., Lennon, J.J., Osborn, A.M., Solan, M., Ven der Gast, C.J.  
1330 & Young, J.P.W. (2007) The role of ecological theory in microbial ecology. *Nature Review*  
1331 *Microbiology*, 5, 384–392.

1332 Proulx, S.R., Promislow, D.E.L. & Phillips, P.C. (2005) Network thinking in ecology and  
1333 evolution. *Trends in Ecology & Evolution*, 20, 345–353.

1334 Puccia, C.J. & Levins, R. (1985) *Qualitative modeling of complex systems: An introduction to*  
1335 *loop analysis and time averaging*, Harvard University Press, Cambridge, MA.

1336 Quezada-Romegialli, C., Jackson, A.L. & Harrod, C. (2018) tRophicPosition: Bayesian Trophic  
1337 Position Calculation with Stable Isotopes. R package version 0.7.5. [https://cran.r-](https://cran.r-project.org/package=tRophicPosition)  
1338 [project.org/package=tRophicPosition](https://cran.r-project.org/package=tRophicPosition)

1339 Rall, B.C., Guill, C. & Brose, U. (2008) Food-web connectance and predator interference dampen  
1340 the paradox of enrichment. *Oikos*, 117, 202–213.

1341 Ramos, R. & González-Solís, J. (2011) Trace me if you can: the use of intrinsic biogeochemical  
1342 markers in marine top predators. *Frontiers in Ecology and the Environment*, 10, 258–266.

1343 Raubenheimer, D. & Simpson, S.J. (2004) Organismal stoichiometry: quantifying non-  
1344 independence among food components. *Ecology*, 85, 1203–1216.

1345 Raubenheimer, D., Simpson, S.J. & Mayntz, D., 2009. Nutrition, ecology and nutritional ecology:  
1346 toward an integrated framework. *Functional Ecology*, 23, 4–16.

1347 Resano-Mayor, J., Hernández-Matías, A., Real, J., Parés, F., Moleón, M., Mateo, R. & Ortiz-  
1348 Santaliestra, M.E. (2016) The influence of diet on nestling body condition of an apex  
1349 predator: a multi-biomarker approach. *Journal of Comparative Physiology B*, 186, 343–362.

1350 Reynolds, D.S., Sullivan, J.C. & Kunz, T.H. (2009) Evaluation of total body electrical  
1351 conductivity to estimate body composition of a small mammal. *Journal of Wildlife*  
1352 *Management*, 73, 1197–1206.

1353 Robinson, B.G., Franke, A. & Derocher, A.E. (2018) Stable isotope mixing models fail to estimate  
1354 the diet of an avian predator. *The Auk*, 135, 60–70.

1355 Rota, T., Jabiol, J., Chauvet, E. & Lecerf, A. (2018) Phenotypic determinants of inter-individual  
1356 variability of litter consumption rate in a detritivore population. *Oikos*, in press, doi:  
1357 oik.05228

1358 Ropert-Coudert, Y. & Wilson, R.P. (2005) Trends and perspectives in animal-attached remote  
1359 sensing. *Frontiers in Ecology and the Environment*, 3, 437–444.

1360 Ruess, L., Schütz, K., Migge-Kleian, S., Häggblom, M.M., Kandeler, E. & Scheu, S. (2007). Lipid  
1361 composition of Collembola and their food resources in deciduous forest stands—  
1362 Implications for feeding strategies. *Soil Biology and Biochemistry*, 39, 1990–2000.

1363 Sadowska, E.T., Baliga-Klimczyk, K., Chrzascik, K. & Koteja, P. (2008) Laboratory model of  
1364 adaptive radiation: a selection experiment in the bank vole. *Physiological and Biochemical*  
1365 *Zoology*, 81, 627–640.

1366 Sander, E.L., Wootton, J.T., & Allesina, S. (2017). Ecological Network Inference From Long-  
1367 Term Presence-Absence Data. *Scientific Reports*, 7, 7154.

1368 Schoepf, I., Pillay, N. & Schradin, C. (2017) The pathophysiology of survival in harsh  
1369 environments. *Journal of Comparative Physiology B*, 187, 183–201.

1370 Schouten S., Klein-Breteler, W.C.M., Blokker, P., Schogt, N., Rijpstra, W.I.C., Grice, K., Baas,  
1371 M. & Sinninghe Damsté, J.S. (1998) Biosynthetic effects on the stable carbon isotopic



1372 compositions of algal lipids: implications for deciphering the carbon isotopic biomarker  
1373 record. *Geochimica et Cosmochimica Acta*, 62, 1397–1406.

1374 Scharnweber, K., Syväranta, J., Hilt, S., Brauns, M., Vanni, M.J., Brothers, S., Köhler, J.,  
1375 Knežević-Jarić, J. & Mehner, T. (2014) Whole-lake experiments reveal the fate of terrestrial  
1376 particulate organic carbon in benthic food webs of shallow lakes. *Ecology*, 95, 1496–1505.

1377 Schulte-Hostedde, A.I., Millar, J.S. & Hickling, G.J. (2001) Evaluating body condition in small  
1378 mammals. *Canadian Journal of Zoology*, 79, 1021–1029.

1379 Schulte-Hostedde, A.I., Zinner, B., Millar, J.S. & Hickling, G.J. (2005) Restitution of mass-size  
1380 residuals: Validating body condition indices. *Ecology*, 86, 155–163.

1381 Sherr, E.B & Sherr, B.F. (2007) Heterotrophic dinoflagellates: a significant component of  
1382 microzooplankton biomass and major grazers of diatoms in the sea. *Marine Ecology*  
1383 *Progress Series*, 352, 187–197.

1384 Simpson, S.J. & Raubenheimer, D. (1993) A multi-level analysis of feeding behaviour: the  
1385 geometry of nutritional decisions. *Philosophical Transactions of the Royal Society B*, 342,  
1386 381–402.

1387 Soong, J.L., Reuss, D., Pinney, C., Boyack, T., Haddix, M.L., Stewart, C.E. & Cotrufo, M.F.  
1388 Design and operation of a continuous  $^{13}\text{C}$  and  $^{15}\text{N}$  labeling chamber for uniform or  
1389 differential, metabolic and structural, plant isotope labeling. *Journal of Visualized*  
1390 *Experiments*, 83, 51117.

1391 Sousa, T., Domingos, T. & Kooijman, S. (2008) From empirical patterns to theory: a formal  
1392 metabolic theory of life. *Philosophical Transactions of the Royal Society B*, 363, 2453–2464.

1393 Sperfeld, E., Wagner, N.D., Halvorson, H.M., Malishev, M. & Raubenheimer, D. (2017) Bridging  
1394 ecological stoichiometry and nutritional geometry with homeostasis concepts and integrative  
1395 models of organism nutrition. *Functional Ecology*, 31, 286–296.

1396 Spitz, J., Ridoux, V. & Brind'Amour, A. (2014). Let's go beyond taxonomy in diet description:  
1397 testing a trait-based approach to prey–predator relationships. *Journal of Animal Ecology*, 83,  
1398 1137–1148.

1399 Steffan, S.A., Chikaraishi, Y., Currie, C.R., Horn, H., Gaines-Day, H.R., Pauli, J.N., Zalapa, J.E.  
1400 & Ohkouchi, N. (2015) Microbes are trophic analogs of animals. *Proceedings of the National*  
1401 *Academy of Sciences*, 112, 15119–15124.

1402 Sternalski, A., Mougeot, F., Eraud, C., Gangloff, B., Villers, A. & Bretagnolle, V., 2010.  
1403 Carotenoids in nestling Montagu's harriers: variations according to age, sex, body condition  
1404 and evidence for diet-related limitations. *Journal of Comparative Physiology B*, 180, 33–43.

1405 Sterner, S.W. & Elser, J.J. (2002) *Ecological stoichiometry: the biology of elements from*  
1406 *molecules to the biosphere*, Princeton University Press, Princeton, NJ, USA.

1407 Stevenson, R.D. & Woods, W.A. (2006) Condition indices for conservation: new uses for evolving  
1408 tools. *Integrative and Comparative Biology*, 46, 1169–1190.

1409 St-John, M.A.S. & Lund, T. (1996) Lipid biomarkers: linking the utilization of frontal plankton  
1410 biomass to enhanced condition of juvenile North Sea cod. *Marine Ecology Progress Series*,  
1411 131, 75–85.

1412 Strauss, T., Gabsi, F., Hammers-Wirtz, M., Thorbek, C. & Preuss, T.G. (2017) The power of  
1413 hybrid modelling: An example from aquatic ecosystems. *Ecological Modelling*, 364 77–88

1414 Tavaré, S., Balding, D.J., Griffiths, R.C. & Donnelly, P. (1997) Inferring coalescence times from  
1415 DNA sequence data. *Genetics*, 142, 505–518.

1416 Thums, M., Fernández-Gracia, J., Sequeira, A.M.M., Eguíluz, V.M., Duarte, C.M. & Meekan,  
1417 M.G. (2018) How big data fast tracked human mobility research and the lessons for animal  
1418 movement ecology. *Frontiers in Marine Science*, 5.

1419 Traugott, M., Kamenova, S., Ruess, L., Seeber, J. & Plantegenest, M. (2013) Empirically  
1420 characterising trophic networks: what emerging DNA-based methods, stable isotope and  
1421 fatty acid analyses can offer. *Advances in Ecological Research*, 49, 177–224.

1422 Trifonova, N., Kenny, A., Maxwell, D., Duplisea, D., Fernandes, J. & Tucker, A. (2015) Spatio-  
1423 temporal Bayesian network models with latent variables for revealing trophic dynamics and  
1424 functional networks in fisheries ecology. *Ecological Informatics*, 30, 142–158.

1425 Urrejola, S., Nespolo, R. & Lardies, M.A. (2011) Diet-induced developmental plasticity in life  
1426 histories and energy metabolism in a beetle. *Revista chilena de historia natural*, 84, 523–533.

1427 Van Oevelen, D., Van den Meersche, K., Meysman, F.J.R., Soetaert, K., Middelburg, J.J. &  
1428 Vézina, A.F. (2010) Quantifying food web flows using linear inverse models. *Ecosystems*,  
1429 13, 32–45.

1430 Vander Zanden, H.B., Soto, D.X., Bowen, G.J. & Hobson, K.A. (2016) Expanding the isotopic  
1431 toolbox: applications of hydrogen and oxygen stable isotope ratios to food web studies.  
1432 *Frontiers in Ecology and Evolution*, 4, 20.

1433 Vanni, M.J. (2002) Nutrient cycling by animals in freshwater ecosystems. *Annual review of*  
1434 *Ecology and Systematics*, 33, 341–370.

1435 Vanni, M.J., Flecker, A.S., Hood, J.M. & Headworth, J.L. (2002) Stoichiometry of nutrient  
1436 recycling by vertebrates in a tropical stream: linking species identity and ecosystem  
1437 processes. *Ecology Letters*, 5, 285–293.

- 1438 Visanuvimol, L. & Bertram, S.M. (2011) How dietary phosphorus availability during development  
1439 influences condition and life history traits of the cricket, *Acheta domesticas*. Journal of  
1440 Insect Science, 11, 1–17.
- 1441 Volterra, V. (1926) Fluctuations in the abundance of a species considered mathematically. Nature,  
1442 118, 558–560.
- 1443 Wallach, A.D., Dekker, A.H., Lurgi, M., Montoya, J.M., Fordham, D.A. & Ritchie, E.G. (2017)  
1444 Trophic cascades in 3D: network analysis reveals how apex predators structure ecosystems.  
1445 Methods in Ecology and Evolution, 8, 135–142.
- 1446 Walsberg, G.E. (1988) Evaluation of a nondestructive method for determining fat stores in small  
1447 birds and mammals. Physiological Zoology, 61, 153–159.
- 1448 Walters, C., Christensen, V. & Pauly D. (1997) Structuring dynamic models of exploited  
1449 ecosystems from trophic mass-balance assessments. Reviews in Fish Biology and Fisheries,  
1450 7, 139–172.
- 1451 Walters, D.M., Fritz, K.M. & Otter, R.R. (2008) The dark side of subsidies: adult stream insects  
1452 export organic contaminants to riparian predators. Ecological Applications, 18, 1835–1841.
- 1453 Walters, W.J. & Christensen, V. (2018) Ecotracer: analyzing concentration of contaminants and  
1454 radioisotopes in an aquatic spatial-dynamic food web model. Journal of Environmental  
1455 Radioactivity, 181, 118–127.
- 1456 Ward, J.E. & Shumway, S.E. (2004) Separating the grain from the chaff: particle selection in  
1457 suspension-and deposit-feeding bivalves. Journal of Experimental Marine Biology and  
1458 Ecology, 300, 83–130.

1459 Weitere, M., Erken, M., Majdi, N., Arndt, H., Norf, H., Reinshagen, M., Traunspurger, W.,  
1460 Walterscheid, A. & Wey, J.K. (2018) The food web perspective on aquatic biofilms.  
1461 Ecological Monographs, in press.

1462 Welti, N., Striebel, M., Ulseth, A., Cross, W., DeVilbiss, S., Glibert, P., Guo, L., Hirst, A., Hood,  
1463 J., Kominoski, J., MacNeill, K., Mehring, A., Welter, J. & Hillebrand, H. (2017) Bridging  
1464 food webs, ecosystem metabolism, and biogeochemistry using ecological stoichiometry  
1465 theory. *Frontiers in Microbiology*, 8. 1298.

1466 Werne, J.P., Baas, M. & Sinninghe-Damsté, J.S. (2002) Molecular isotopic tracing of carbon flow  
1467 and trophic relationships in a methane-supported benthic microbial community. *Limnology  
1468 and Oceanography*, 47, 16.

1469 Wilder, S.M., Raubenheimer, D. & Simpson, S.J. (2016) Moving beyond body condition indices  
1470 as an estimate of fitness in ecological and evolutionary studies. *Functional Ecology*, 30, 108–  
1471 115.

1472 Winemiller, K.O. (1990) Spatial and temporal variation in tropical fish trophic networks.  
1473 *Ecological Monographs*, 60, 331–367.

1474 Woodward, G., Perkins, D.M. & Brown, L.E. (2010) Climate change and freshwater ecosystems:  
1475 impacts across multiple levels of organization. *Philosophical Transactions of the Royal  
1476 Society B*, 365, 2093–2106.

1477  
1478

1479 Figure's Captions:

1480 Fig. 1 (A) Multiple correspondence analysis showing the general distribution of empirical methods  
1481 used in trophic ecology (see Table 1 for data and abbreviations). Arrows indicate the affinity of the  
1482 methods with the selected features: organisational level (from individuals to ecosystems), ecological  
1483 questions (interaction vs. flux of energy/matter), or commitment of budget, time and efforts (investment,  
1484 price). Observational scale (i.e. microscopic to macroscopic) was mostly discriminated by axis 4, not  
1485 shown here, but scores are in appendix. (B) Subjective classification of trophic models based on their  
1486 affinity with the organisational level and ecological question (after C. Gaucherel and E. Thebault, pers.  
1487 com.). ODE: ordinary differential equation, IBM: individual-based model, DEB: dynamic energy and mass  
1488 budget.

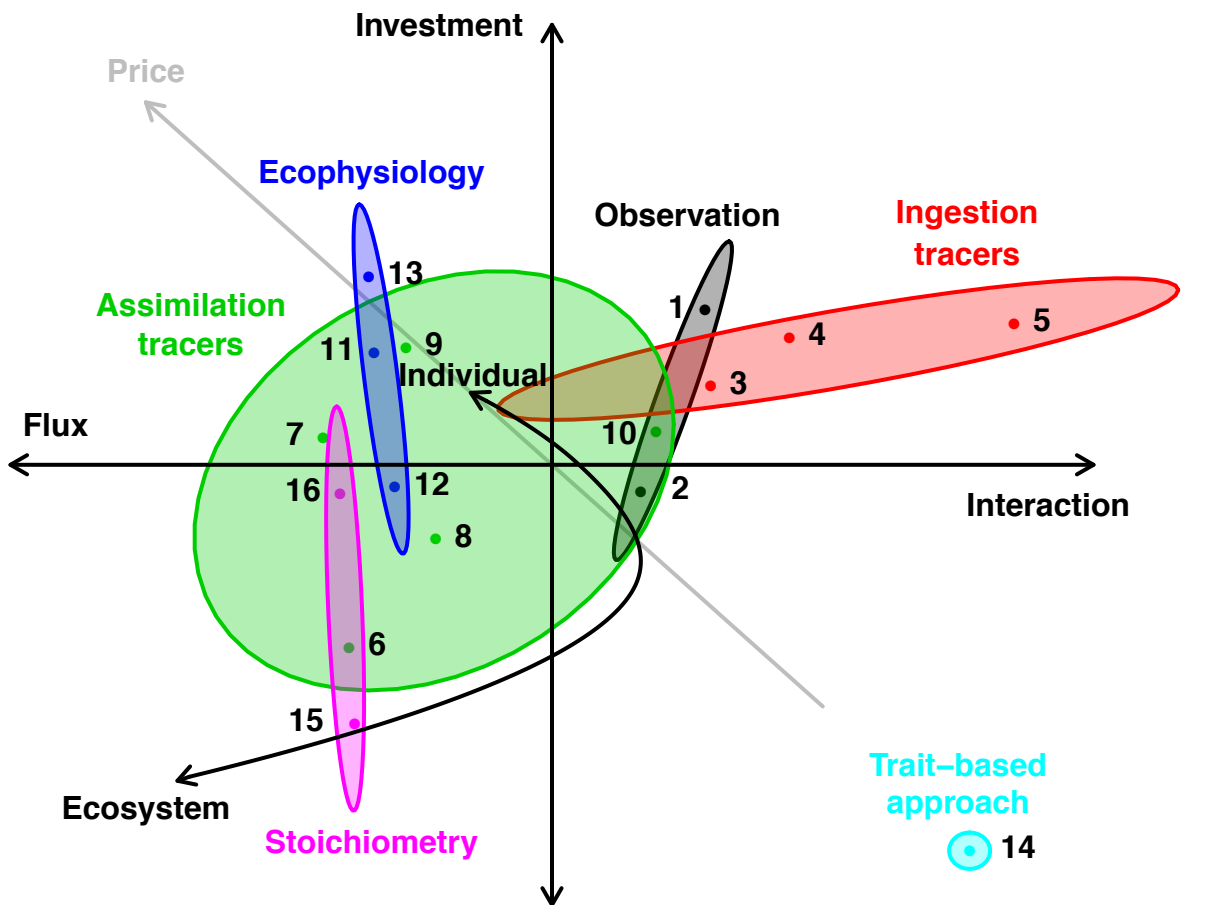
1489

1490

1491 Table 1. Synthesis of the main advantages and drawbacks of several methods commonly used in trophic  
1492 ecology. Expert assessment of empirical methods according to 12 variables is also shown: price, investment  
1493 (is it a turnkey method? or does it require further development?), observational scale (from microscopic to  
1494 large organisms), organisational level (from individuals to ecosystem), type of ecological question  
1495 (interaction vs. flux of energy/matter), and type of produced data (qualitative vs. quantitative). 0 indicates  
1496 "not adapted" and 1 indicates "strong affinity". The exceptions are the variables "price" and "investment",  
1497 where 0 and 1 stand for "low" and "high" respectively, and the "data" variable, where 0 and 1 stand for  
1498 "qualitative" and "quantitative" data respectively.

Figure 1

**A**



1: Observation of feeding activity

6: Bulk stable isotope analysis

11: Body composition

2: Incubation experiments

7: Isotopes as labels

12: Body condition index

3: Gut & feces content

8: Fatty Acids

13: Body fluid biochemistry

4: Gut DNA

9: Compound-SIA

14: Trait-based approach

5: Parasites as biological tags

10: Contaminants

15: Ecological stoichiometry

16: Resource quality

Figure 1

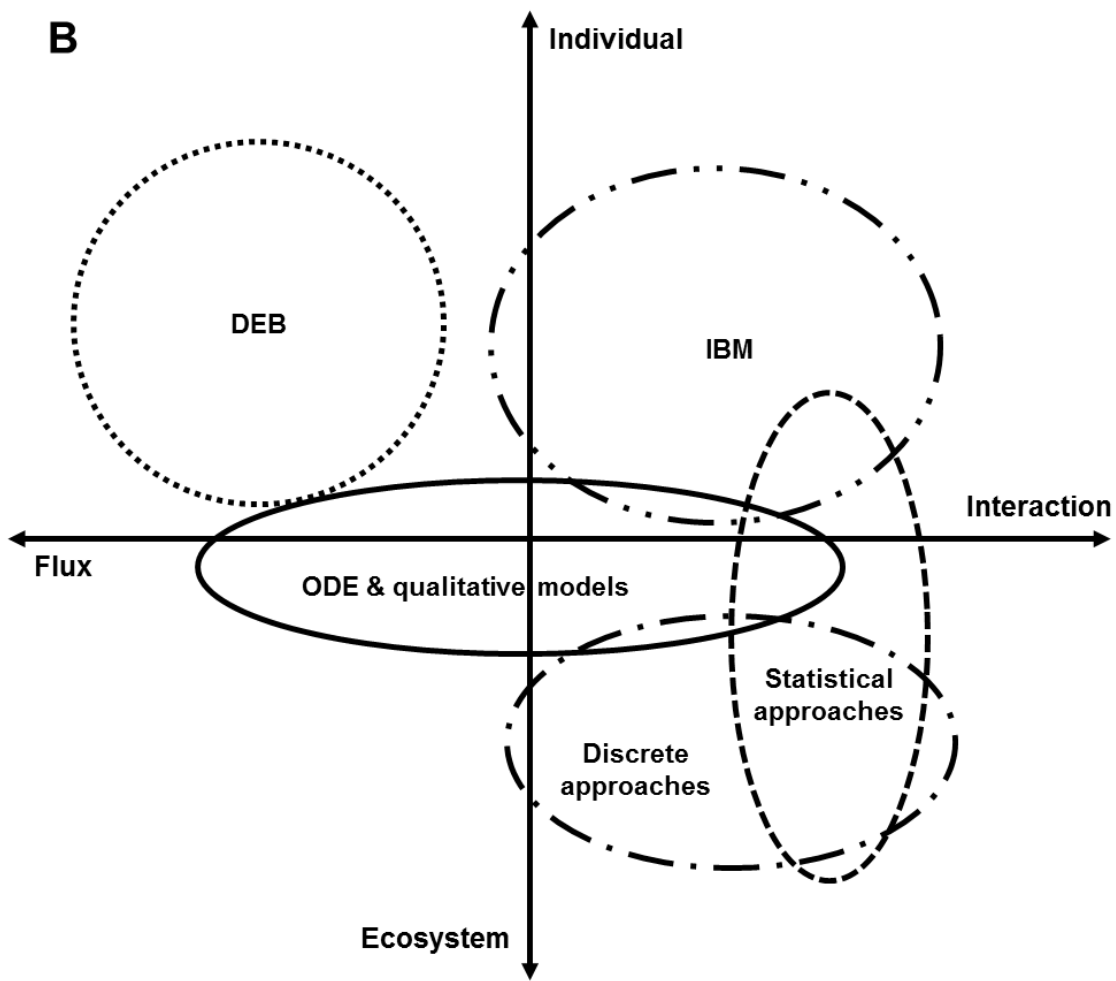




Table 1 - Empirical and experimental methods for trophic ecology

Detecting prey-predator relationships, or food sources	Price	Investment	Micro	Small	Large	Individual	Population	Community	Ecosystem	Interaction	Flux	Data
	Observation of feeding activity	0	1	0	1	1	1	1	0	0	1	0
Incubation experiments	0	1	1	1	0	1	1	1	0	1	1	1
Gut & feces content	0	1	1	1	1	1	1	0	0	1	0	1
Gut DNA	1	1	0	1	1	1	1	1	0	1	0	0
Parasites as biological tags	0	1	0	0	1	1	1	1	0	1	0	0
Bulk Stable Isotope Analysis	0	0	0	1	1	1	1	1	1	0	1	1
Detecting prey-predator relationships, or food sources	Price	Investment	Micro	Small	Large	Individual	Population	Community	Ecosystem	Interaction	Flux	Data
	Isotopes as labels	1	1	0	1	1	1	1	1	0	1	1
Fatty Acids	1	1	1	1	1	1	1	1	1	0	1	0
Compound-Specific Isotopic Analysis	1	1	0	1	1	1	1	1	0	0	1	1
Contaminants	1	1	1	1	1	1	1	1	0	1	1	0

Table 1 - Empirical and experimental methods for trophic ecology

Quantifying the consequences of feeding from individuals to ecosystems	Price	Investment	Micro	Small	Large	Individual	Population	Community	Ecosystem	Interaction	Flux	Data
Body composition	1	1	1	1	1	1	1	0	0	0	1	1
Body condition indices	0	0	0	1	1	1	1	0	0	0	1	1
Body fluid biochemistry	1	1	0	1	1	1	1	0	0	0	1	1
Traits	0	0	1	1	1	0	1	1	0	1	0	0
Ecological Stoichiometry	0	0	1	1	1	1	1	1	1	0	1	1
Resource quality	1	0	1	1	1	1	1	0	0	0	1	1

Table 1 - Empirical and experimental methods for trophic ecology

Advantages	Drawbacks
Straightforward and well-established / Relatively cheap / Various interactions and scales (incl. intraspecific variability) / <i>In situ</i> or under controlled conditions / Large scale records of feeding-behaviors are possible with miniaturization of loggers	Temporal & spatial scales are design-dependent / Difficult to measure assimilation
Effect-size of specific variables / Suitable for filter-feeders	Mostly laboratory-based, so deviation from natural conditions might occur
Well-established / Relatively cheap / Information on nature and size of ingested food items	Snapshot of diet (does not reveal interaction strength) / Time consuming / Labile tissues or prey might be overlooked
Identification of gut microbiomes / Works on partly digested, poorly recognizable, food items	Expensive / Susceptible to typical drawbacks associated to molecular approaches
Host specificity / Integration of diet over long time periods / Potential information on diet provenance	Lethal / No information on prey size / Needs empirical knowledge of life-cycles (and potentially biogeography) of parasites / Bias due to side-effects of parasites on hosts' health and behavior
Well-established / Relatively cheap / Traces the carbon and nitrogen food sources in consumers / Important replication is possible / suited for <i>in situ</i> as well as laboratory studies	Turnover times and isotopic discrimination might vary among species, tissues and the environment studied / Not well-suited when a large number of potential resources are considered
Advantages	Drawbacks
Trace the fate (timing and scale) of carbon and nitrogen from microbes to macroscopic consumers / Differentiation between N and C uptake from unlabelled C and N pools	Difficult to provide quantitative fluxes / Needs to know turnover incorporation and routing in tissues (e.g. issue of uniformly labeling the food source) / Can be relatively expensive depending on the scale or nature of the labeling
Well-established / Traces flux and nutritional quality	Non-specific biomarkers / Needs <i>a priori</i> knowledge of producers' and consumers' metabolism / relatively expensive
Integrative tracers when coupled with a labeling / Target analysis promising in Microbial Ecology / Ideally it can trace limiting compounds and eventually detect minor food sources unseen by classical methods / Better knowledge in fractionation and turnover times for specific compounds	Needs a good understanding of the biochemical and physiological pathways in investigated organisms or tissues / Relatively expensive
Might trace spatio-temporal ingestion and assimilation depending on the persistence of compounds within tissues (e.g. lipophilic contaminants might biomagnify along food chains) / Potentially information on foraging area, and on contamination status of species and ecosystems	Needs a source of contamination / Biases due to side-effects on health and behavior / Needs knowledge of contaminants' biogeochemical cycle, distribution and persistence in organisms and in the environment / Relatively expensive

Advantages	Drawbacks
Measuring energy storage is one of the most direct and quantitative indicator of body condition	Dissection might be time-consuming and tedious / Alternative techniques are less invasive but more expensive and require further development
Cheap / Estimation of energy storage / Applicable <i>in situ</i> and in the lab from various data sources / Can be non-lethal (allometry) and applied on endangered wildlife	Indirect measure, non-specific marker of trophic interactions / Large temporal variations (e.g. depending on annual and circadian cycle)
Integrative assessment of physiological state (health, diet deficiencies)	Physiological state can be affected by other factors than nutritional stress / Difficult interpretation that needs integrated assessment (combination of markers) / Expensive / Less suitable on small animals
Cheap / Morphological repositories widespread / Adapted for paleo-trophic ecology / Useful to infer the dimension of trophic niches	Difficult to assess strength in species interactions / Little understanding of how traits are inter-correlated / Little consensus and practical guidance in methodology
Cheap / Permits to scale up processes from molecules to ecosystems, from physiology to ecosystem functioning / Predictive	Cannot be used as a tracer of biomass / Sometimes too general and missing important information (e.g. nature of C compounds)
Essential compounds are key drivers of diet selection along the life of an organism / Existence of a "nutritional geometry" framework	Young field of research / Needs integrative, and thus potentially expensive, measures

Table 1 - Modelling methods for trophic ecology

Connecting species within networks from ecological data (inference methods)	Advantages
Gaussian graphical models	Popular framework to infer interactions among continuous variables / Obtained precision matrix is a direct proxy of network structure
Bayesian networks & dynamic Bayesian networks	Adapted to class-occurrence data / Suitable to represent directed interactions (trophic, parasitic...) / Can be used to infer ecosystem static states or dynamics / Numerous heuristics are available to reach efficient approximate inference
Logic-based approaches	Adapted to qualitative data / Can incorporate background (expert) knowledge on the interactions / Describes directed interactions / Requires relatively less data than others
Linear inverse inference	Particularly suited to infer the magnitude of missing interactions based on simple assumptions
Approximate Bayesian computation	Replaces the step of likelihood computation by simulations of the model with variable parameter values
Testing the implications of trophic interactions (trophic models)	Advantages
Ordinary Differential Equations and qualitative models	Understanding of indirect interactions and species dynamics in food webs / Qualitative models useful when the quantification of each trophic interaction is unreachable / Useful to investigate potential consequences of perturbations on food web functioning and stability
Statistical approaches	Can be built from simple field observations of individual occurrences / Useful to evaluate management strategies dedicated to increase food web resilience (operational models)
Discrete approaches	Adapted to discrete and qualitative interactions / Well adapted to large interaction networks, whatever the nature of components and processes / No need of calibration, except the expert knowledge defining the model / Relevant to deduce all possible trajectories and stabilities the system may experience.
Individual-based models	Help understanding how patterns emerge from inter-individual interactions / Able to scale up from trait-based interactions to structure of ecological networks
Dynamic energy and mass budget approaches	Model the quantitative facets of metabolism by depicting rules of allocations and processes at the organism level / Application to diet reconstructions

Table 1 - Modelling methods for trophic ecology

Drawbacks
The network is undirected (unlike trophic/parasitic relations) / High computational complexity (sparse matrix inversion) / Unsuitable for classes-occurrence observations / Assume a gaussian distribution of interactions
Not adapted to continuous observations / Exact inference is out of reach given the combinatorial nature of the underlying optimization problem.
The learnt network describes "deterministic" interactions / Accuracy of learning is hard to validate because the learning process is rather "black box"
Relies on a known network topology and on the static assumption of mass balance between all network compartments at equilibrium.
Requires substantial computing time
Drawbacks
ODE models for large food webs often require a disproportionate number of parameters, and all parameter combinations are impossible to test through sensitivity analysis / Predictions of the effects of perturbations with qualitative models become ambiguous for large networks (< 15 nodes)
Not process-based, thus no mechanistic understanding / Dependant on collected data (over- and under-fitting)
Hardly adapted to continuous quantitative abundances, biomasses or fluxes in the network
Often require very intensive numerical simulations (calibration and validation stages) / Not adapted to heavy individual or multiscale networks / Hardly provide rigorous and demonstrated analyses
Huge personal investment required / Decreasing interest when used for complex applications at community or ecosystem levels