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Are food web structures well represented in isotopic spaces?

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Summary

1. Isotopic analyses are increasingly used to assess the structure of food webs and a series of isotopic functional indices have been proposed in the last decade to characterize this structure. These indices are based on the foundational assumption that proximity in the isotopic space informs on trophic similarity between species. While it has been recognized for long that this simplifying assumption should be used with caution, no formal evaluation of its domain of validity has been performed to date.

2. We here simulate a large number (15,000) of food webs with varying characteristics to assess i) whether isotopic distance is a good proxy of trophic dissimilarity; ii) whether isotopic functional indices are good proxies of trophic functional properties; and iii) how the quality of these two proxies depend on various species and food web properties.

3. We first demonstrate that isotopic distance is moderately correlated with trophic similarity in simulated food webs. We find however that two isotopic community metrics investigated (*Rao*'s index of functional divergence and *NND* index of functional originality) are good indicators of community trophic structure, especially in food webs with large connectance, and when predators have low levels of diet specialization.

4. We also find that isotopic functional indices are decreasingly reliable as they aim at characterizing more subtle functional patterns (such as *sdNND*, an index of between species variation in originality).

5. Taken together, our results suggest that the reliability of the isotopic mapping of consumers decreases with their number of potential prey species, but increases with their number of realized prey species. Consequently, isotopic

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reliability decreases in species-rich food webs with low connectance, large vertical diversity and large incidence of omnivory.

6. Our study highlights that researchers should not hope to grasp subtle patterns of food web structure based solely on widely used isotopic indices. They should instead envisage mobilizing such isotopic information in combination with complementary data types.

Key-words Connectance, isotopic functional indices, niche model, omnivory, virtual ecology.

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Introduction

Since its formalization, the niche concept has captured the attention of numerous evolutionary biologists and ecologists (Vandermeer 1972; Chase & Leibold 2003). After Hutchinson's (1957) concept, the ecological niche is commonly defined as a hyper-volume in the *n*-dimensional space of ecological variables, within which a species can maintain and develop its population. It builds upon Grinnell's (1917) niche concept, in which responses and performances of species depend on environmental resources, but also upon Elton's (1927) niche concept which introduces species effects on their environment that later led to niche construction theory (Laland, Matthews & Feldman 2016). As a result, Elton's (1927) niche concept of the role of a species in a community has led to the definition of the trophic niche (Vandermeer 1972, Schoener 2009) or the functional niche (Chase & Leibold 2003). Many tools and complementary approaches have been used to characterize species niches, usually by measuring their relation with a set of environmental variables (e.g. the thermal constraints of grassland ant species, Albrecht & Gotelli 2001) or with a set of morphological traits (e.g. bill sizes in birds, Radford & Du Plessis 2003; or mouth aperture in fishes, Hjelm, Persson & Christensen 2000). Proxies linked with the feeding ecology of a species give access to an estimate of its trophic niche while abiotic parameters are more related to its habitat niche (Guisan & Zimmermann 2000), with both trophic and habitat niches contributing to the species niche.

Among the analytical tools to characterize trophic niches, ecologists have developed the use of stable isotopes with the primary goals of reconstructing species diet and determining the contribution of producers and trophic pathways in food web communities (Fry 2006; Boecklen et al. 2011). Stable isotope analyses (SIA) - and

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particularly ^{13}C and ^{15}N - provide quantitative measurements of diet items and a temporal integration of the food that is actually assimilated (West et al. 2006). Newsome et al. (2007) further proposed to transform the *n*-dimensional functional Eltonian space into a 2-dimensional δ-space. The idea of the isotopic δ-space - also called the isotopic niche - as a quantitative measurement of the trophic niche has since become a cornerstone of most SIA studies. In this vein, many ecologists emphasize "that the isotopic niche is distinct from, but in many circumstances should align closely with, aspects of the actual trophic niche (e.g. particular resource pools utilized or relative trophic position within a web)" (Layman et al. 2012). This concept of isotopic niche has therefore been used first at the population level (e.g., Hentschel 1998; Bolnick et al. 2003; Gelpi et al. 2013; Yeakel et al. 2016). Isotopic analyses have been subsequently used at the community level, with species positions in an isotopic δ-space being considered as proxies of food web trophic structures (Layman et al. 2007).

Statistical tools have been developed for quantifying the overall structure of species niches in a community of interacting species. Layman et al. (2007) suggested using a set of metrics to provide measurements of the relative spacing of species in a δ-space. Among them, the mean nearest neighbor distance (*NND*) was suggested as a measure of species packing and thereby of trophic redundancy, while the standard deviation of nearest neighbor distance (*sdNND*) was suggested to measure the among species variation in their trophic originality. Then, many empirical studies have tried to grasp changes in community trophic structure based on community isotopic metrics, such as in African estuaries following the wet season (Abrantes, Barnett & Bouillon 2014), in 5 coastal marine benthic habitats after colonization by an engineer species (Rigolet et al.

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2015), in littoral fish communities following species invasions (Fanelli et al. 2015) or in several human-created salt-marches over a long-term period (Nordstrom et al. 2015). Other metrics have since been used for trophic function, such as the *Rao*'s index of quadratic entropy that is a measure of functional divergence (Schleuter et al. 2010; Rigolet et al. 2015). Building upon metrics designed to quantify the functional diversity of community (i.e. functional richness, evenness and redundancy, Villéger, Mason & Mouillot 2008; Mouillot et al. 2013), other more-refined isotopic functional indices have since been applied to communities of freshwater fishes (Cucherousset & Villéger 2015) or marine invertebrates (Rigolet et al. 2015).

Ecological inferences from such community-wide metrics require caution to avoid pitfalls (Hoeinghaus & Zeug 2008) and can be sensitive to outlying isotopic compositions or to sample size (Jackson et al. 2011; Brind'Amour & Dubois 2013). In particular, community-wide stable isotope analyses are based on two untested assumptions: 1) that two close species in an isotopic δ-space have similar functional role in the food web, and 2) that isotopic metrics are good proxies of food web structural properties. The first assumption is known to be problematic, since several distinct consumer diets can in theory produce similar isotopic signatures (Phillips & Gregg 2003; Hoeinghaus & Zeug 2008). However, no formal analysis of the severity of this potential limitation has been performed for realistic food webs, so that it is still unclear whether this assumption is tenable or not in general. In the same perspective, community-wide isotopic metrics are obviously noised representations of food web structure. But the magnitude of this noise has not been thoroughly investigated, so that it is unclear how trusty such metrics can be for real-world applications. The aim of the present study is thus to precisely assess the validity of these assumptions with

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simulations of realistic food webs with variable properties. We specifically investigate here i) whether isotopic proximity is a good proxy of trophic similarity in general, ii) whether community-wide isotopic metrics are good proxies of food web structural properties, and iii) how the quality of these two proxies depend on various species and food web properties, including diet specialization, species richness of consumers and primary producers, and food web connectance.

Materials and methods

The general flowchart of the methodology is presented in Figure 1. It contains four main steps: i) the simulation of realistic food webs using the niche model (Williams & Martinez 2000), ii) the computation of a matrix of pairwise trophic similarity between consumer species for each simulated food web, iii) the computation of isotopic biplots for each food web, based on simple rules of isotopic discrimination, and iv) the computation of two sets of functional diversity indices, based either on species distributions within isotopic biplots, or on the pairwise matrix of trophic similarity.

Food web simulation

We simulated food webs using the niche model (Williams & Martinez 2000) that has been shown to yield realistic food web structures and has been extensively used in food web modelling (Allesina, Alonso & Pascual 2008). The niche model consists in attributing to each of the S species a niche position η_i and a feeding range within a single niche axis. Trophic links are then randomly drawn inside the feeding ranges of the various species. We followed the simulation procedure described in Allesina et al. (2008). For each simulated food web, species that did not have prey were considered to be primary producers, while the others were considered to be consumers. Using this

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procedure, we simulated food webs of varying species richness S in {10; 15; 20; 25; 30; 40; 50; 60; 80; 100} and of varying target connectance C_{target} in {0.1; 0.15; 0.2; 0.25; 0.3}. The connectance is a measure of the network complexity, defined as the fraction of all possible links that are realized in a network (Gardner & Ashby 1970). These parameter ranges were chosen to encompass the range of food web structures commonly reported in the literature (e.g., Williams & Martinez 2000; Dunne 2006).

We considered three alternative rules to attribute the trophic link strengths $\boldsymbol{F}_{ij}$ between a predator i and a prey j in the simulated food webs. The first simulated rule consisted in equally sharing trophic link strengths among the prey of a predator, so that the trophic links between a predator and its k prey species had a strength equal to $1/k$. The two other simulated rules considered two different levels of diet specialization (hereafter noted s) with s in {0.6; 0.9}. Under these rules, each predator preferentially preyed upon one of its prey species (randomly selected), with trophic link strength equal to s, and we divided the remaining trophic strength $1\text{-}s$ equally among its other prey species.

Finally, species abundances $\boldsymbol{B}_i$ (defined as total species biomass, that is the average individual biomass multiplied by species numerical abundance) were randomly drawn in a lognormal distribution with mean equal to 0 and standard deviation equal to 3 on a log scale, to recover realistic abundance distributions (Cohen, Jonsson & Carpenter 2003). Our simulation protocol enables to generate food webs with variable distribution of species number and abundances across trophic levels, from pyramidal to inverted pyramidal food webs, this variability being observed among real food webs (Cohen et al. 2003; Turney & Buddle 2016).

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We further constrained simulated food webs to match a number of criteria that lead to the exclusion of i) food webs having more than seven trophic levels, in accord with empirical ranges (McGarvey, Dowling & Cohen 2016), ii) food webs with a single primary producer or less than four consumer species to avoid trivial isotopic biplots, and iii) food webs with multiple unconnected components (since the niche model can generate such food webs). For each parameter and rule combination, we simulated 100 food webs matching our inclusion criteria, leading to a total of 15,000 simulated food webs (10 species richness x 5 target connectance x 3 interaction rules x 100 replicate simulations). We further performed a complementary analysis with constant species abundances that lead to qualitatively similar results (Fig. S6).

Computation of trophic similarity

For each simulated food web, we computed the trophic similarity between all pairs of consumers using the flow similarity index $\boldsymbol{Sim_{flow}}$ proposed by Yodzis and Winemiller (1999). This index is computed on realized feeding link strengths, with the trophic similarity between two consumer species *i* and *j* given by: $\boldsymbol{Sim_{flow}}(i,j) = \Sigma_k (\boldsymbol{F}_{ik} \cdot \boldsymbol{B}_k \cdot \boldsymbol{F}_{jk} \cdot \boldsymbol{B}_k) / \mathrm{Sqrt}[\Sigma_k (\boldsymbol{F}_{ik}^2 \cdot \boldsymbol{B}_k^2) \cdot \Sigma_k (\boldsymbol{F}_{jk}^2 \cdot \boldsymbol{B}_k^2)]$ where the sum (denoted by Σ_k) is performed on all the indices *k* of prey consumed by species *i* and *j*, where $\boldsymbol{F}_{ik}$ is the trophic link strengths between predator *i* and prey *k*, and where $\boldsymbol{B}_k$ is the abundance of prey *k*. In the following, we will make use of dissimilarity measures, defined as $\boldsymbol{d}(i,j) = 1 - \boldsymbol{Sim_{flow}}(i,j)$. To assess whether our results were robust to our measure of trophic similarity, we additionally used a second index of trophic similarity $\boldsymbol{Sim_{cor}}$ that is based on correlations: $\boldsymbol{Sim_{cor}}(i,j) = \mathrm{cor}(\boldsymbol{F}_{i.} \cdot \boldsymbol{B}, \boldsymbol{F}_{j.} \cdot \boldsymbol{B})$ where $\boldsymbol{F}_{i.}$ is the vector of trophic strengths between predator *i* and all other species and $\boldsymbol{B}$ is the vector of

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species abundances. Results with this second index of trophic similarity were qualitatively similar and are therefore presented as supplementary material (Fig. S7).

Computation of isotopic biplots

For each weighted food web, we simulated the associated biplot in the isotopic space ($\delta^{13}C$, $\delta^{15}N$). To do this, we assigned to each primary producer a $\delta^{13}C$ value randomly drawn from a uniform distribution between -30 ‰ and -10 ‰. This range of $\delta^{13}C$ values for primary producers is consistent with empirical ranges (Boutton 1991). Furthermore, the random draw of $\delta^{13}C$ values for producers enables to generate food webs with variable $\delta^{13}C$ ranges and with variable isotopic proximities between primary producers, characteristics that have been previously shown to potentially alter isotopic characterizations of food webs (Hoeinghaus & Zeug 2008). We attributed to all primary producers a $\delta^{15}N$ value equal to 0 ‰, thereby neglecting among producers variations in $\delta^{15}N$ values. Although primary producers do have $\delta^{15}N$ signatures that vary in space and time, primary consumers dampen such variations and are generally characterized by similar $\delta^{15}N$ values (Cabana & Rasmussen 1996; Post 2002). Consequently, while our assumption of constant $\delta^{15}N$ values among primary producers is clearly crude, its consequence of a constant $\delta^{15}N$ values among primary consumers is much less crude. The assumption of a constant $\delta^{15}N$ values among primary consumers is the one that matters for the analyses reported here that are based on consumer species.

We then attributed to consumers isotopic values equal to the weighted average of the isotopic values of their prey plus a constant discrimination value equal to 1 ‰ for $\delta^{13}C$ and 3.5 ‰ for $\delta^{15}N$. These discrimination values are consistent with empirical

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findings (Vander Zanden & Rasmussen 2001; Post 2002; Vanderklift & Ponsard 2003). More precisely, the isotopic values ($\boldsymbol{\delta^{13}C_i}$, $\boldsymbol{\delta^{15}N_i}$) of consumer *i* were determined by solving the following linear system of equations:

$$\boldsymbol{\delta^{13}C_i} = 1 + \Sigma_j \boldsymbol{F_{ij}} \,.\, \boldsymbol{B_j} \,.\, \boldsymbol{\delta^{13}C_j} / \Sigma_j \boldsymbol{F_{ij}} \,.\, \boldsymbol{B_j} \quad (1)$$

$$\boldsymbol{\delta^{15}N_i} = 3.5 + \Sigma_j \boldsymbol{F_{ij}} \,.\, \boldsymbol{B_j} \,.\, \boldsymbol{\delta^{15}N_j} / \Sigma_j \boldsymbol{F_{ij}} \,.\, \boldsymbol{B_j} \quad (2)$$

In the following, we used untransformed isotopic values to compute matrices of isotopic dissimilarity between consumer species that were based on Euclidean distances between species isotopic positions, and to compute isotopic functional indices (see next section). We obtained very similar results when performing a standardization of isotopic values prior to these computations. Corresponding results using a Z score standardisation on each isotopic axis (Hoeinghaus & Zeug 2008) are reported in Fig. S8.

For each simulated food web, we computed the pairwise correlation between the matrices of trophic and isotopic dissimilarity between species (excluding the diagonal of the matrices). We further computed these same correlations when considering only the half most basal or top species, and the half most specialist or generalist species, where the level of generalism was assessed by the number of prey for a given species.

To quantitatively characterize simulated food webs, we computed a number of summary statistics: the connectance *C* of the food web (that may slightly differ from the target connectance C_{target} used to simulate the food web); the number *Npp* of primary producers; the Gini-Simpson index *G* of abundances evenness (G=1-$\Sigma_k \boldsymbol{B_k} \,.\, \boldsymbol{B_k} / B_{tot}^2$); the ranges of δ^{13}C and δ^{15}N in the food web; the minimal isotopic distance

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$\min(NND_{pp})$ between two primary producers ($\min(NND_{pp})=\min_{i\neq j}\{e_{ij}\}$, where e_{ij} is the Euclidean distance between species i and j in the isotopic space, Fig. 1); the mean trophic height *mth* in the food web ($mth= \Sigma_k \mathbf{TP_k}/S$, where $\mathbf{TP_k}$ is the trophic position of species k and *S* is species richness); an index of mean omnivory in the food web using the omnivory index o'_k of Bersier, Banasek-Richter & Cattin (2002); and two indices to characterize whether the food web is rather pyramidal or inverted pyramidal (Turney & Buddle 2016): the species bias in trophic position (TP) defined as the difference between the mean trophic height (mth) and the trophic height centre (equal to 1 + 0.5 × (max TP -1), to account for the fact that the basal trophic position equals 1 instead of 0) and the biomass-based bias in trophic position for which each species is weighted by its relative abundance in the computation of the mean trophic height. Negative biases in TP indicate a pyramidal food web. Trophic positions were defined from species isotopic values in $\delta^{15}N$ with the formula $TP = 1 + \delta^{15}N / 3.5$. This isotopic-based computation of trophic positions takes into account the loops in the food webs. The distributions among simulated food webs of these various characteristics are reported in Fig. S1 in Supporting Information, as well as their pairwise correlations (Table S2). We further assessed whether simulated food webs had realistic characteristics by comparing them to compilations of empirical food webs. We found that simulated ranges of connectance levels included empirical ones (Fig. S3a) and that simulated proportions of basal, intermediate and top species were in line with empirical ones (Fig. S3b-d). Other food web characteristics have been less systematically documented in empirical studies, thereby rendering comparisons difficult. Overall, we followed simulation procedures that have been abundantly used in the literature for producing realistic food webs (Williams & Martinez 2000; Allesina, Alonso & Pascual 2008).

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Functional indices

We computed three functional indices on the isotopic biplots: *Rao*'s index of quadratic entropy (Rao 1982) that is a measure of isotopic functional divergence (Mason et al. 2005; Schleuter et al. 2010, Fig. 2a,b), and the mean and standard deviation of nearest neighbour distances (*NND* and *sdNND* respectively, Layman et al. 2007). *NND* and *sdNND* are measuring the functional originality of assemblages (Mouillot et al. 2013, Fig. 2c-f). *Rao*'s index is weighted by biomass, while *NND* and *sdNND* are not (Rigolet al., 2015). Although NND and sdNND are correlated (Fig. S4), each index encapsulates different aspects of the trophic structure (Fig. 2). Even though new isotopic functional indices have been suggested (Cucherousset & Villéger 2015; Rigolet et al. 2015), we focused on *Rao*, *NND* and *sdNND* indices as they can be computed from a pairwise distance matrix. This particularity enabled us to also compute these metrics on the matrices ***d*** of trophic dissimilarity. We designated in the following the metrics computed on isotopic biplots by Rao_i, NND_i and $sdNND_i$, while those computed on trophic dissimilarity matrices by Rao_t, NND_t and $sdNND_t$.

Results

Is isotopic proximity indicative of trophic similarity?

We obtained moderate correlations between trophic dissimilarity and Euclidean distance in the isotopic space (Fig. **3**a). Correlations were larger among the half most basal species than among species at the top of the food web. They were also larger among the half most generalist species compared to the half most specialist ones (Fig. 3a). We further found that correlations were larger in food webs with larger connectance (Fig. 3b), lower species-richness (Fig. 3c), when predators fed equally

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among their different prey species (Fig. 3d), and when primary producers were more segregated in the isotopic space (Fig. 3e). These correlation levels were also slightly impacted by other characteristics of the simulated food webs (Fig. 4): correlation levels notably decreased with species abundance evenness (measured by the Gini-Simpson diversity index), with the level of omnivory in the food web, and with the vertical diversity of the food web (measured by the range in $\delta^{15}N$). Note that we used partial correlations in this last analysis to account for correlations among the various characteristics of simulated food webs (Table S2).

Are isotopic community patterns indicative of community trophic structure?

We found variable levels of correlations between functional indices computed on isotopic biplots and those computed on trophic dissimilarity matrices (Fig. 5). *Rao*'s index of functional divergence was the one presenting the largest correlation levels (Cor = 0.79, Fig. 5a), followed by the *NND* index of functional originality (Cor = 0.72, Fig. 5b) and by the *sdNND* index of among species variability in functional originality (Cor = 0.47, Fig. 5c). Similar trends were obtained when constant species abundances were used in the food web simulations (Cor = 0.63, 0.48 and 0.16 respectively, Fig. S6), and when the correlation-based index of trophic dissimilarity was used (Cor = 0.79, 0.74 and 0.51 respectively, Fig. S7). Furthermore, standardization of isotopic values prior to the analysis only brought very marginal changes in correlation levels for the first two metrics (Cor = 0.83, 0.74 and 0.47 respectively, Fig. S8) and very similar results were obtained when simulated food webs were filtered to have characteristics within documented empirical ranges (Cor = 0.79, 0.73 and 0.48 respectively, Fig. S9). Finally, the ability of isotopic functional indices to capture trophic properties was little affected by the characteristics of

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simulated food webs (Table S5). The most notable effects were that i) Rao_i was less correlated with Rao_t in more even food webs, ii) NND_i was less correlated with NND_t in food webs with larger connectance, omnivory and mean trophic height, and in inverted pyramidal food webs, and iii) $sdNND_i$ was less correlated with $sdNND_t$ in food webs with larger omnivory, species richness and mean trophic height.

Discussion

Should I trust my isotopic biplot?

Our results suggest that species proximity in an isotopic biplot is substantially correlated with trophic similarity, although with a sizeable level of noise (Fig. 3). The fact that species with different trophic regimes can have similar isotopic signatures has been recognized early on as a potential limitation of analyses of isotopic community structure (Layman et al. 2007; Hoeinghaus & Zeug 2008). More precisely, Hoeinghaus and Zeug (2008) demonstrated that different food web trophic structures could be associated with similar values of community functional metrics, if the sources of these food webs have different isotopic positions. Our study is, to our knowledge, the first to extensively assess the magnitude of these limitations using simulated realistic food webs. Our results suggest that isotopic proximity is a better indicator of trophic similarity in smaller (Fig. 3c) and more densely connected (Fig. 3b) food webs, when predators have low levels of diet specialization (Fig. 3d), and when primary producers are more segregated in the isotopic space (Fig. 3e). We also found that the effects of other food web characteristics on the quality of isotopic proxies were rather low (Fig. 4). Still, we were able to evidence a negative impact of vertical diversity (number of trophic levels) on the correlation between isotopic and trophic similarity (Fig. 4). This finding suggests that isotopic approaches may be

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slightly more reliable in terrestrial than in pelagic food webs that contain a larger number of trophic levels in general (McGarvey et al. 2016). Such generalization must however be made with caution, given the large variability in trophic structures encountered within biomes (Schoener 1989). For instance, a general comparison of the reliability of isotopic approaches in benthic and pelagic food webs is challenging, since benthic food webs tend to harbour less trophic levels (Schoener 1989) but larger levels of omnivory (Kopp et al. 2015) than pelagic ones. These two types of variation in trophic structure are likely to have compensatory influences on the isotopic reliability within these two food web types (Fig. 4).

Interestingly, our simulations suggest that despite the fact that isotopic biplots provide a blurred representation of trophic similarity between species, widely-used functional indices based on such biplots reasonably grasp the main characteristics of food web trophic structure (Fig. 5). Indeed, we found that isotopic metrics of functional divergence (*Rao*) and mean functional originality (*NND*) were remarkably strongly correlated with the same metrics based on trophic dissimilarities (Fig. 5a,b). The signal to noise ratio was nevertheless progressively lower as more subtle community trophic patterns were assessed with isotopic indices, so that among species variability in trophic originality was less well captured by isotopic indices (*sdNND*, Fig. 5c). These results did not change when a standardization of isotopic values was performed prior to community-level analyses (Fig. S8). While our approach did not enable us to assess the consistency of other widely-used functional indices (Cucherousset & Villéger 2015; Rigolet et al. 2015), our results should prompt ecologists to restrain the use of functional indices measuring very subtle patterns of food web structure that may be less adequately captured by isotopic biplots.

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Taken together, our results suggest that the reliability of the isotopic mapping of consumers decreases with their number of *potential* prey species. Indeed, we found that isotopic reliability decreased with the total number of species and their abundances evenness (Gini-Simpson, Fig. 4). Furthermore, isotopic reliability also decreased when considering only species located at the top half of the food web (Fig. 3a). These top consumers have a larger number of potential prey than basal species. Consistently, we also found that isotopic reliability decreased with vertical diversity (range in $\delta^{15}N$) and mean trophic height (Fig. 4). The same mechanics may also explain the negative impact of omnivory on isotopic reliability (Fig. 4), since increasing omnivory in the food web also means increasing the number of potential prey for consumers. Such a negative effect of the number of potential prey species would also recall the fact that there is a stronger indeterminacy of diet reconstruction techniques as the number of potential prey species increases (Phillips 2001). At the same time, our results suggest that the reliability of the isotopic mapping of consumers increases with their number of *realized* prey species. Indeed, we found that isotopic proxies were more reliable for generalist consumers (Fig. 3a). We speculate that the isotopic signal of generalist consumers may be more efficiently smoothed, so that remaining isotopic differences between such consumers may be more reliably interpreted as trophic differences rather than as long-lasting effects of minor isotopically original food sources. The fact that connectance was found to positively impact the quality of isotopic proxies (Fig. 3b) is also consistent with this result, since increasing connectance will mechanistically increase the incidence of generalist species.

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Our simulation procedure enabled us to study communities harbouring variable ranges in $\delta^{13}C$ from 1.8 to 25.2 ‰ (Fig. S1f), and variable minimal isotopic distances $\min(NND_{pp})$ between primary producers from 0.001 ‰ to 10 ‰ (Fig. 3e). Total ranges in $\delta^{13}C$ were found to have little impact on trophic-isotopic correlations (Fig. 4), a result consistent with the fact that standardizing isotopic values leads to qualitatively similar results (Fig. S8). These last two findings indicate that the absolute range of isotopic values has a lower impact on the results than the relative isotopic positions of the various species of the food web. Logically, when several primary producers have close isotopic signatures (i.e., low $\min(NND_{pp})$), isotopic distance between consumer species tends to be a less good indicator of trophic dissimilarity (Fig. 3e). Still, our results suggest that the effect of primary producers isotopic proximity on trophic-isotopic correlations is limited, and clearly lower than the one of other food web characteristics such as connectance (Fig. 4). As a result, the inclusion of observation noise in this simulation framework should not alter much our results since this would solely noise isotopic biplots without changing their overall structure that is more strongly controlled by other food web characteristics.

We necessarily had to make a number of simplifications in our simulations. For instance, we did not take into account the spatial and temporal variabilities of isotopic signatures of food sources (e.g., Post 2002; Flaherty & Ben-David 2010), discrimination variability (e.g., Vanderklift & Ponsard 2003; Boecklen et al. 2011; Emmery et al. 2011; Hussey et al. 2014) nor intraspecific variability (Araújo, Bolnick & Layman 2011) and temporal variability in trophic position of consumers (Dalerum & Angerbjörn 2005; Cherel et al. 2007). Assessing the impacts of releasing each of these simplifications is beyond the scope of this study. We however contend that these

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simplifications should not alter much the main conclusions of our study. They are indeed much more likely to affect the intra-population variance than the inter-population variance of isotopic positions, the latter being the main focus of community-wide analyses examined in this contribution. Besides, stable isotope techniques provide a temporal averaging of consumer diets and of temporal variabilities of isotopic signatures of food sources, although with variable time scales of integration depending on trophic position (O'Reilly et al. 2002) and on the tissue studied (Crawford, McDonald & Bearhop 2008). This implies that community wide isotopic analyses will provide a temporally averaged view of food web structure. Still,

441 temporal variability at larger time scales does exist (Dalerum & Angerbjörn 2005; Cherel et al. 2007) and calls for extending snapshot investigations of isotopic structure of food webs towards diachronic data collection protocols (Warren 1989; Berg & Bengtsson 2007). A potentially more problematic simplification is to have considered that all primary producers had equal $\delta^{15}N$ isotopic values, while both temporal and interspecific variations in $\delta^{15}N$ have been reported in the literature for primary producers (Cabana & Rasmussen 1996; Post 2002). To account for this variability, it has been proposed to use nitrogen baselines of primary consumers (Cabana & Rasmussen 1996) that show much less temporal and interspecific variations (Post 2002). Since our analyses are based on the consumers of the food webs, they are actually performing this same control of the nitrogen baselines. In other words, while our assumption of constant $\delta^{15}N$ values among primary producers is clearly crude, it generates primary consumers with constant $\delta^{15}N$ values. This model output is much more realistic (Cabana & Rasmussen 1996; Post 2002) and is the one that matters for the analyses reported here that are based on consumer species.

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Perspectives to strengthen the robustness of isotopic analyses at the community level

Our study provides a theoretical support for the cautionary use of (simple) isotopic functional indices to characterize food web trophic structure. It however stresses severe limitations of this approach if one wants to characterize subtle changes in food web structure. Our study therefore calls for more refined analytical strategies to fully realize the promises of isotopic community analyses. Different propositions have been made in the literature to strengthen isotopic analyses at the community level that we here recall and comment in the light of our simulation findings.

The first approach consists in using mixing models to translate the isotopic δ-space into a dietary p-space prior to performing functional analyses (Newsome et al. 2007). This proposed strategy has been mainly envisioned at the scale of trophic modules rather than at the scale of entire food webs (Newsome et al. 2007). Recent progress in mixing model methodology enables to assess dietary uncertainties and to mobilize complementary information to constrain dietary reconstructions at the scale of entire food webs (Kadoya et al. 2012). It is thus now possible to reconstruct pairwise trophic similarity matrices using such methodologies. It should thus be possible to compute distance-based functional indices from these matrices, as we did in the present study. Such an approach will however require a good prior knowledge of the food web topology, in order to get a reliable reconstruction of the weighted trophic links.

The second approach consists in mobilizing complementary information to get a finer understanding of food web structure, either by increasing the number of isotopes studied (e.g., Phillips & Gregg 2003, Swanson et al. 2015) or by including other types of data such as stomach content (Harrigan, Zieman & Macko 1989), faeces content

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(Codron et al. 2005), DNA metabarcoding (Evans et al. 2016; Pornon et al. 2016), abundance distribution and prior knowledge (Kadoya et al. 2012) or experimental results (Dubois et al. 2007). Indeed, these various types of data offer complementary insights on food web functioning and may therefore be efficiently combined. Powerful statistical tools have been developed to integrate multiple lines of evidence in a single framework (Moore & Semmens 2008). These tools still need to be generalized at the scale of entire food webs (but see Kadoya et al. 2012 for a promising realization). Modern statistical tools, such as approximate Bayesian computation and other inverse techniques might enable to calibrate food web models from a wider array of data types and in a more flexible way (Beaumont 2010, Jabot & Bascompte 2012; Melian et al. 2015). By enabling a better reconstruction of trophic matrices, they should contribute to a better understanding of the subtle functional characteristics of food webs that direct isotopic functional indices are unlikely to document.

More generally, our study provides a theoretical and mechanistic foundation to assess the strength and weaknesses of isotopic pattern analyses that are increasingly performed. It points out that isotopic functional indices are likely to be sensible indicators of major characteristics of food web structure, but that they are increasingly noised as one seeks to document more subtle patterns. The approach employed here to benchmarking pattern analyses on mechanistically simulated data is increasingly used in ecological science (Zurell et al. 2010), but still deserve more attention in the field 3 of trophic ecology. This study contributes to filling this gap.

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Author contributions statement

All authors conceived the ideas and designed methodology; FJ conducted the analyses; FJ and SD led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data Accessibility

- R script to perform simulations: uploaded as online supporting information (Appendix S10).

- Full simulation results: uploaded as online supporting information (Appendix S11).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1: Supplementary figures and tables. 3

Appendix S9: R script to perform simulations: uploaded as online supporting 4 information.

Appendix S10: full simulation results.

Figures

Figure 1. Flowchart of the analyses. (a) Empirical food webs are simulated with a set of varying properties (i.e. number of species, connectance, specialization) using the niche model (Williams & Martinez 2000). (b) For each simulated food web, a 1 corresponding isotopic biplot is computed using a constant ^{15}N and ^{13}C prey-predator discrimination factor. (c,e) One matrix of trophic dissimilarities ($\mathbf{d}_{ij}$) and one matrix of Euclidian distances ($\mathbf{e}_{ij}$) are then computed from each simulated food web and each corresponding isotopic biplot, respectively. (d) The correlation between these two sets of indices is computed for each simulated food web to test the assumption that isotopic proximity is a good proxy of trophic similarity. (f) Two sets of functional metrics are calculated on both matrices of trophic dissimilarities (Rao_t, NND_t, $sdNND_t$) and of isotopic distances (Rao_i, NND_i, $sdNND_i$), and correlations between trophic and isotopic functional metrics are investigated. This second analysis serves to assess the ability of isotopic metrics to capture community trophic structure.

Figure 2. Illustration of istopic diversity metrics. (a,b) Rao index quantifies the isotopic functional divergence (i.e. spread of communities) and is weighted by biomass. When abundant species are located near the centroid (a), and/or when

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species are close in the isotopic space, Rao is low. (c,d) NND quantifies the average proximity of the neighbouring species in the isotopic space. When pairs of species are close in the isotopic space (c), NND is low, while when species are regularly spaced (d), NND is large. (e,f) sdNND quantifies the variability in proximity of neighbouring species. When some pairs of species are close while other pairs of species are far from each other (f), sdNND is large.

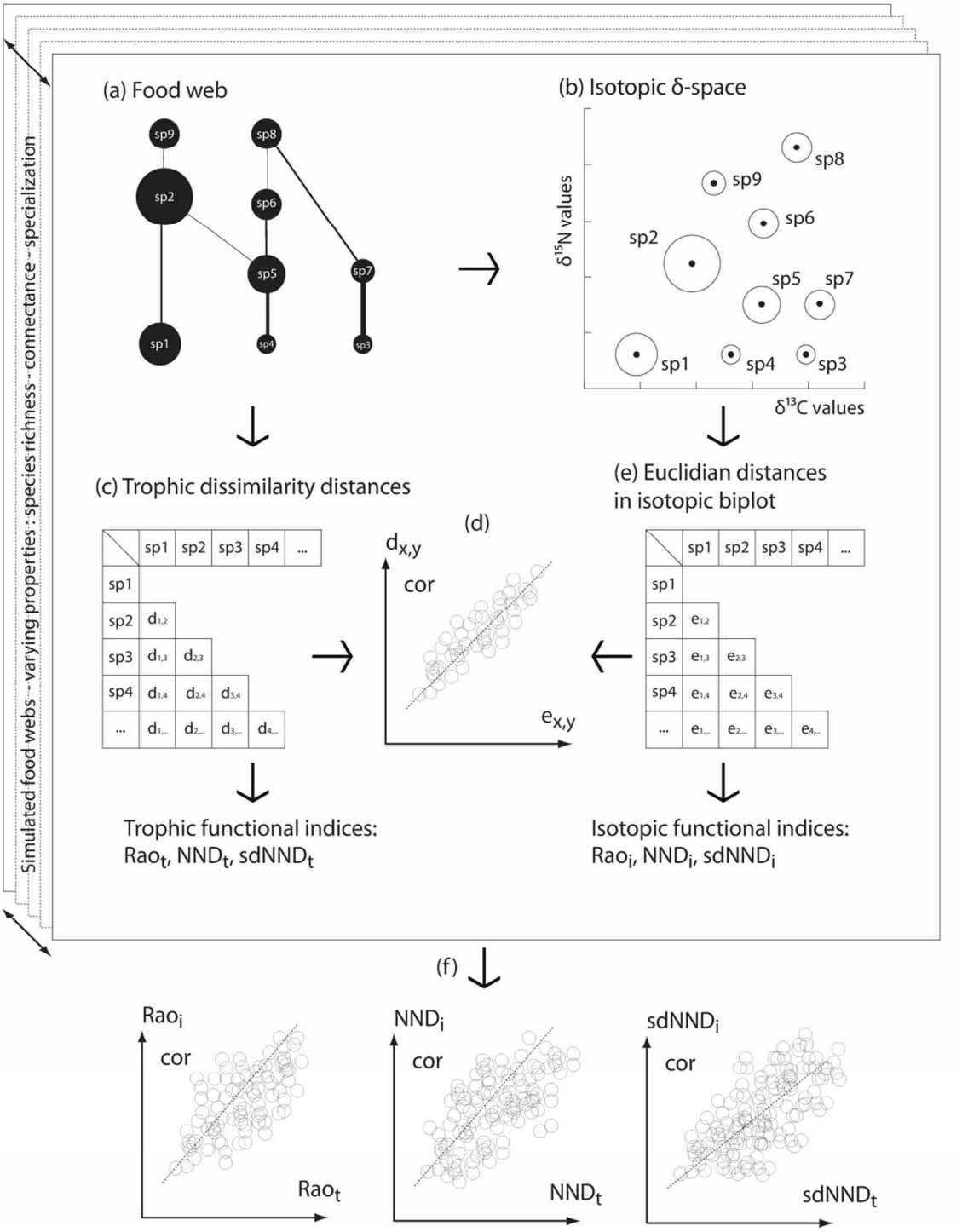
Figure 3. Correlations between trophic and isotopic dissimilarity in simulated food webs. (a) Correlation levels between trophic dissimilarities $\mathbf{d}_{ij}$ and isotopic distances $\mathbf{e}_{ij}$ vary depending on the kind of species included in the analysis. (b,c,d) Effects of simulated food web connectance, species richness and diet specialization on correlation levels.

Figure 4. Effects of the characteristics of simulated food webs on trophic-isotopic correlations.

Figure 5. Correlations between isotopic and trophic diversity metrics.

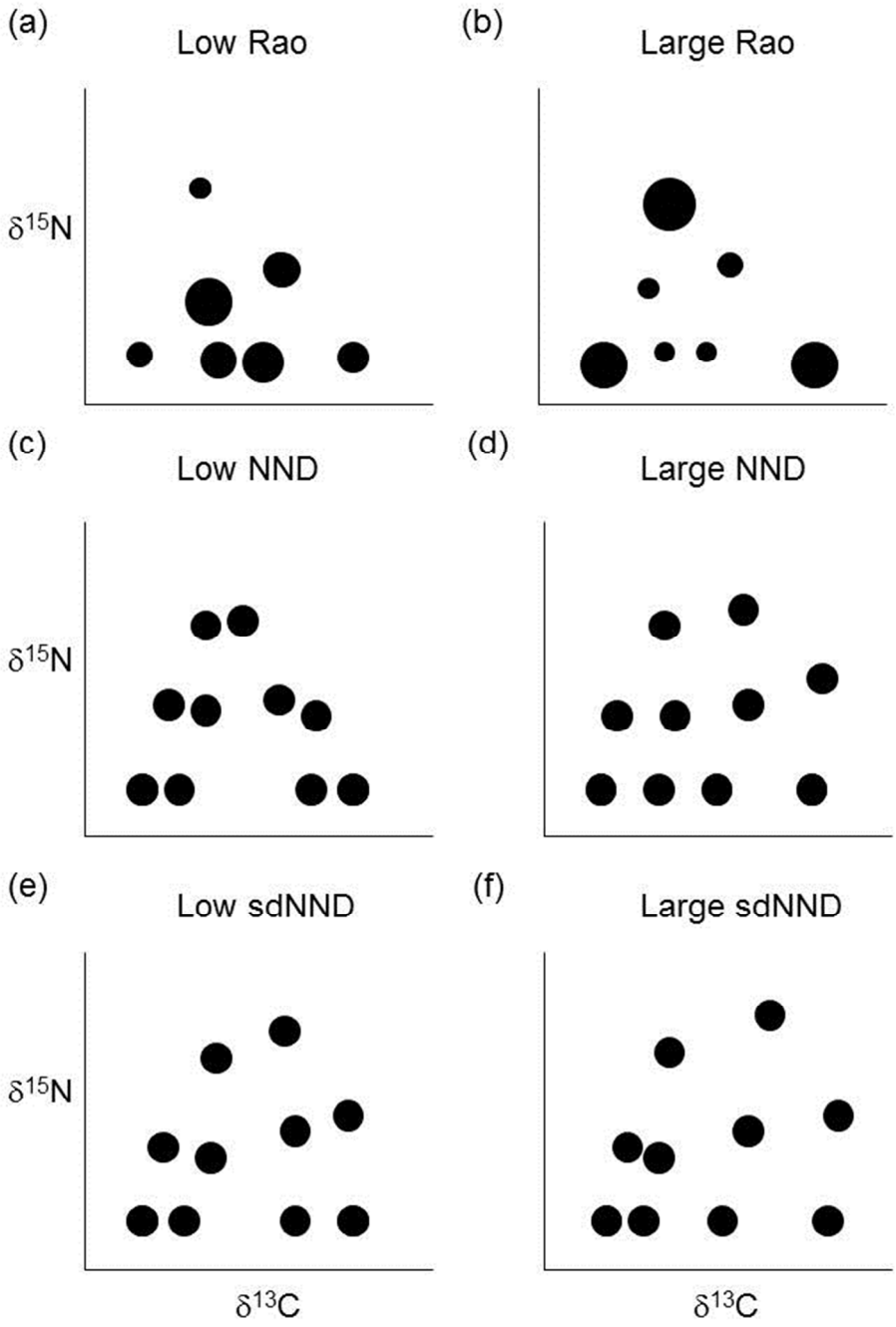
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Figure 1.



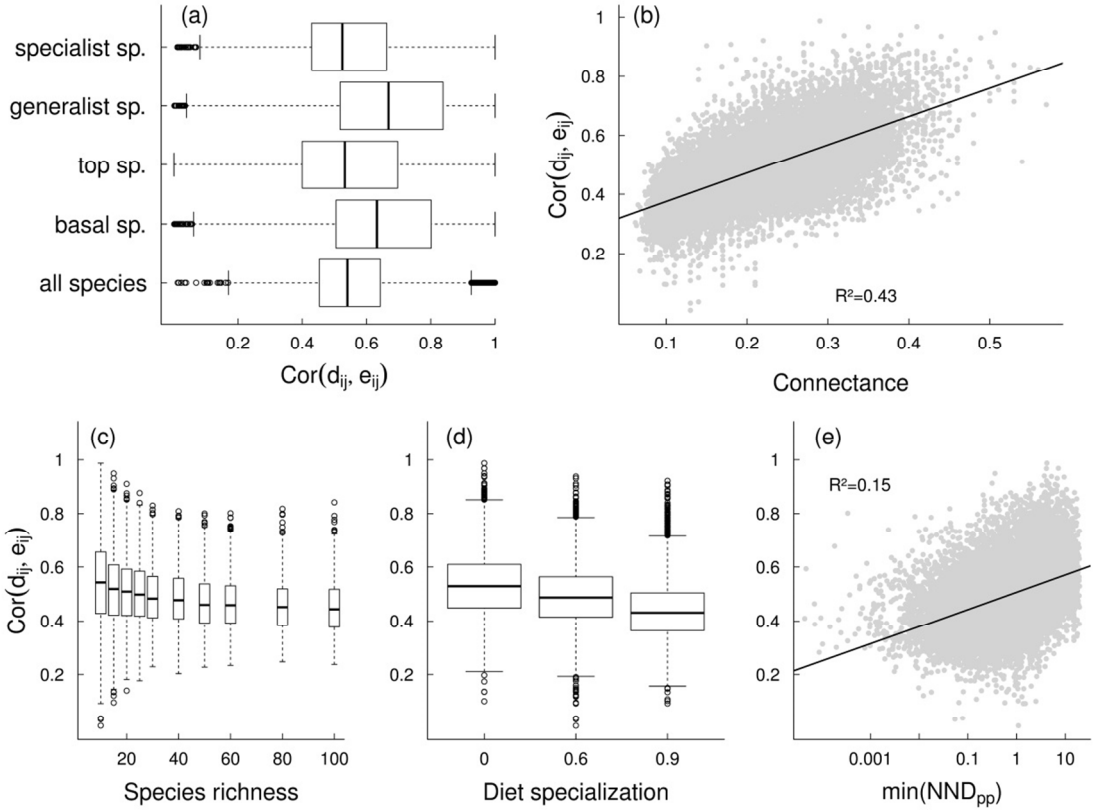
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Figure 2.



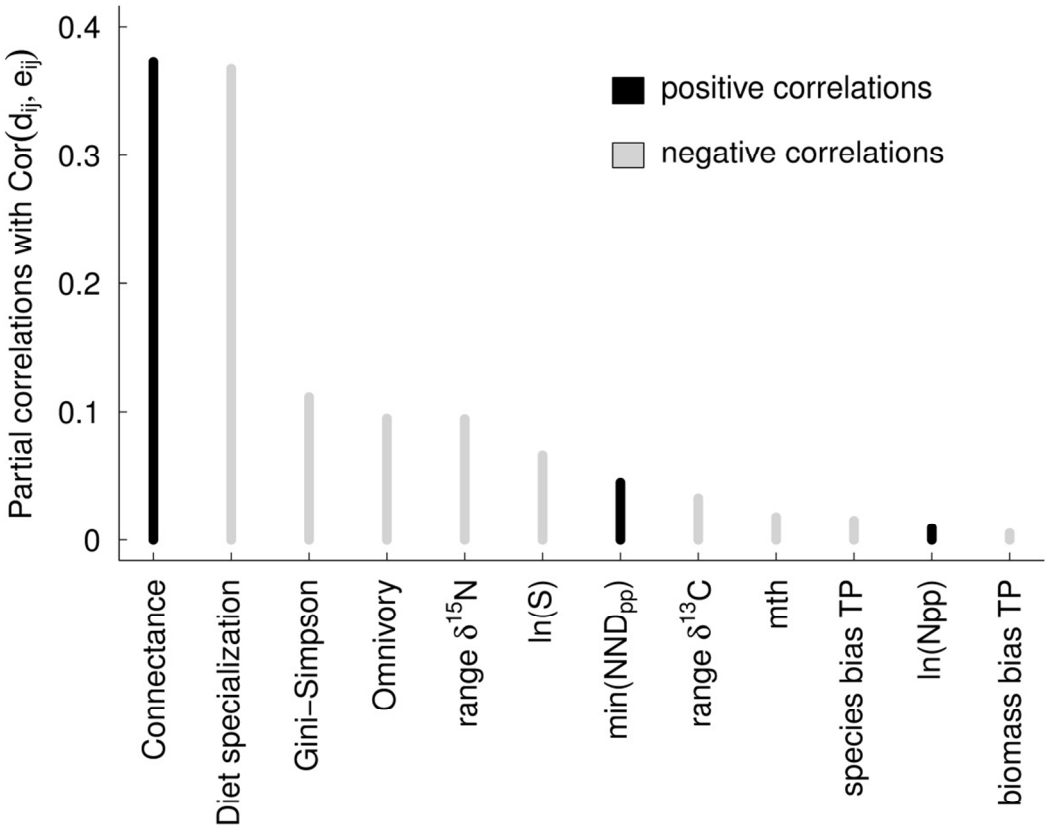
Author-produced version of the article published in Functional Ecology, 2017, 31(10), 1975-1984.
The original publication is available at http://www.sciencedirect.com/
doi : 10.1016/j.ecss.2008.08.013

Figure 3.



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doi : 10.1016/j.ecss.2008.08.013

Figure 4.



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The original publication is available at http://www.sciencedirect.com/
doi : 10.1016/j.ecss.2008.08.013

Figure 5.

