Inferring species interactions in ecological communities: a comparison of methods at different levels of complexity

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Summary

1. Natural communities commonly contain many different species and functional groups, and multiple types of species interactions act simultaneously, such as competition, predation, commensalism or mutualism. However, experimental and theoretical investigations have generally been limited by focusing on one type of interaction at a time or by a lack of a common methodological and conceptual approach to measure species interactions.

2. We compared four methods to measure and express species interactions. These approaches are, with increasing degree of model complexity, an extinction-based model, a relative yield model and two generalized Lotka-Volterra (LV) models. All four approaches have been individually applied in different fields of community ecology, but rarely integrated. We provide an overview of the definitions, assumptions and data needed for the specific methods and apply them to empirical data by experimentally deriving the interaction matrices among 11 protist and rotifer species, belonging to three functional groups. Furthermore, we compare their advantages and limitations to predict multispecies community dynamics and ecosystem functioning.

3. The relative yield method is, in terms of final biomass production, the best method in predicting the 11-species community dynamics from the pairwise competition experiments. The LV model, which is considering equilibrium among the species, suffers from experimental constraints given the strict equilibrium assumption, and this may be rarely satisfied in ecological communities.

4. We show how simulations of a LV stochastic community model, derived from an empirical interaction matrix, can be used to predict multispecies community dynamics across multiple functional groups.

5. Our work unites available tools to measure species interactions under one framework. This improves our ability to make management-oriented predictions of species coexistence/extinction and to compare ecosystem processes across study systems.

Key-words: asymptotic stability, community dynamics, demographic stochasticity, ecological network, equilibrium, experimental uncertainties, functional groups, interaction strength, interaction matrix, non-additive effects

Introduction

Understanding the nature of species interactions in biological communities is a longstanding issue in ecology and conservation biology (Gause 1934; MacArthur & Levins 1967; May 1972; Levine & Hiller Ris Lambers 2009), and many explanations for the high biodiversity observed in natural communities have been proposed (Chesson 2000; Williams & Martinez 2000; Hubbell 2001; Holyoak, Leibold & Holt 2005; Allesina, Alonso & Pascual 2008). A key aspect of all of these perspectives is the understanding of species interactions, which dates back to pioneering theoretical and empirical work (Volterra 1926; Gause 1934). Therefore, the characterization of the interaction matrix built on all pairwise species interactions may allow the understanding of the complexity and dynamics of multispecies communities. Theoretical work showed that important properties of the interaction matrix, such as the variance and the mean of the interaction terms, dictate the maximum number of species in a community, as well as its stability (Berlow 1999; Kokkoris *et al.* 2002). Specifically, recent theoretical studies proved that both the architecture of the ecological networks and the distribution of interaction strengths impact ecosystem stability (Allesina & Tang 2012; Loreau & de Mazancourt 2013).

Measuring interactions among species in natural communities is thus a formidable and commonly conducted task in community ecology (Laska & Wootton 1998; McCann 2000;

Berlow *et al.* 2004). For example, experiments were performed on interacting species in controlled environments in order to test theoretical predictions on the relationship between diversity and stability. However, representative experiments on grasslands (Roxburgh & Wilson 2000; Dormann & Roxburgh 2005), insects (Ayala, Gilpin & Ehrenfeld 1973), molluscs (Paine 1992), hydra species (Case & Bender 1981) or in microbial communities (Vandermeer 1969; McGrady-Steed, Harris & Morin 1997) have not only been giving contrasting results on the relationship between diversity and stability in biological communities (McCann 2000; Ives & Carpenter 2007), but were also based on different approaches on how to quantify species interactions. The use of different methods to establish interaction coefficients in these studies thus interferes with the interest of identifying common mechanisms. In other words, are the differences reported across system artefacts due to the different methods applied or do they reflect biological differences?

We compared and reviewed four commonly adopted methods that are used to infer species interactions. We use laboratory microcosm experiments (see Altermatt *et al.* 2015) for an empirical comparison and complemented them with stochastic simulation models, in order to give a methodological framework which covers a wide spectrum of possible types of species interactions and which can be applied across most types of study systems. We studied the local coexistence of protist communities composed of different functional groups (see Supporting Information Appendix S1 for a detailed information of how the functional groups were derived). Our species are competing for the same resources in homogeneous and steady physical environments (i.e. no spatial structure in the environment and no temporal fluctuations across the experimental time period). In our model system, composed of 10 protists and one rotifer species belonging to three functional groups, different forms of species interactions, such as competition for the same resources (Foster & Bell 2012), interference competition (Amarasekare 2002), predator–prey dynamics, and in principle even positive relationships, such as cooperation or mutualism (Bulleri, Bruno & Benedetti-Cecchi 2008), can be expected and have been observed in previous works (Holyoak & Sachdev 1998; Fox & McGrady-Steed 2002; Jiang & Morin 2005; Altermatt *et al.* 2011; Carrara *et al.* 2015a).

We characterized the interaction matrix of all possible 55 pairwise species combinations with the following methods: the first, called 'Extinction' method (*EX*), ranks the species balancing the number of extinctions of a species against all the others (Cadotte *et al.* 2006). The second method ('Relative Yield' method, *RY*), often used in the biodiversity-ecosystem functioning (BEF) literature (Loreau *et al.* 2001), weights the population performance of a species in the presence of a competitor, compared to an isolation treatment. The third ('Lotka-Volterra Equilibrium' = *EQ*) and the fourth methods ('Lotka-Volterra Dynamics' = *LVD*) are based on generalized Lotka-Volterra (LV) models (see, e.g., Kokkoris *et al.* 2002): *EQ* is assuming equilibrium (Dormann & Roxburgh 2005), whereas *LVD* is also taking the temporal dynamics into account (see Materials and Methods). Table 1 gives an overview of the precise definitions, assumptions and data needed for the specific methods. All four methods are individually widely used in both diversity-functioning and predator–prey research (see, e.g., Ayala, Gilpin & Ehrenfeld 1973; Case & Bender 1981; Paine 1992; Tilman *et al.* 2001; Dormann & Roxburgh 2005; Cadotte *et al.* 2006; Perkins, Holmes & Weltzin 2007; Haddad *et al.* 2008; Violle *et al.* 2011), but to our knowledge, they have not yet been compared under a consistent experimental and theoretical framework (see also Ives, Cardinale & Snyder 2005) and on the same empirical data set.

Here, we aimed (i) at measuring species interactions across multiple functional groups in a common framework that can be applied to other systems as well; (ii) at comparing different analytical methods commonly used to describe species interactions; and finally (iii) at using our results to suggest a method on how pairwise interaction coefficients can be used to predict multispecies coexistence and productivity. We eventually make recommendations on best practices and method-of-choice when measuring and calculating species interactions with the goal of predicting species' persistence and community composition.

Materials and methods

AQUATIC COMMUNITIES

We conducted laboratory microcosm experiments to quantify species interactions in a highly standardized way. Such experiments have been

Table 1. Comparison of four methods describing species interactions (*EX* = extinction, *RY* = relative yield, *EQ* = Lotka-Volterra equilibrium, *LVD* = Lotka-Volterra dynamics). The table describes the methods, gives the mathematical formula to derive them and lists the type of data needed to calculate them (r = intrinsic growth rate, K = carrying capacity, time series, long-term experiment). In parenthesis, non-compulsory data. The prime indicates quantities rescaled by the carrying capacities

Method	Description	Formula	r	K	Time series	Long term
'*EX*'	Balancing the number of extinctions in competition trials	$\alpha_{ij}^{EX} = \sum_{\mathrm{rep}=1}^{6} \left(\delta_{\phi_{i|j;0}^{\star}} \right)$	–	–	–	√
'*RY*'	Reduction of population density relative to carrying capacity	$\alpha_{ij}^{RY} = \phi_{i|j}^{*}/K_i - 1$ $(\alpha_{ij}^{RY})' = n_{i|j}^{*} - 1$	–	√	–	√
'*EQ*'	Fitting two-species interaction terms (by Lotka-Volterra equations at equilibrium) using single-species parameters	$\alpha_{ij}^{EQ} = (\phi_i^* - K_i)/\phi_j^*$ $(\alpha_{ij}^{EQ})' = (n_i^* - 1)/n_j^*$	(√)	√	–	√
'*LVD*'	Fitting two-species interaction terms time-series data (by Lotka-Volterra equations, using single-species parameters)	α_{ij}^{LVD}: best fit from eqn (3) $(\alpha_{ij}^{LVD})'$: best fit from eqn (4)	√	√	√	(√)

a useful and general tool to test theoretical predictions in ecology and evolution (McGrady-Steed, Harris & Morin 1997; Fox & McGrady-Steed 2002; Fukami & Morin 2003; Caddotte *et al.* 2006; Haddad *et al.* 2008; Carrara *et al.* 2012, 2015a; Livingston *et al.* 2012). Specifically, they allow the identification of causalities and bridging the gap between the complexity of natural systems and the level of abstraction inherent to all theoretical models (Holyoak & Lawler 2005). Importantly, they allow the complete characterization of all species interactions and can thus give an overall picture needed for our comparison. All experiments were done following commonly available laboratory protocols (for details, see Altermatt *et al.* 2015).

A set of 10 protist and one rotifer species belonging to three functional groups was used in our experiments (henceforth referred to as protists). The three functional groups were based on size (Giometto *et al.* 2013) and trophic status of the protists and included small protists, large protists and mixotrophs. The functional grouping was done following Petchey & Gaston 2002, and extensive details on the derivation and description of the functional groups are given in the Supporting Information file and Fig. S1. The species were as follows: *Chilomonas* sp., *Colpidium* sp., *Cyclidium* sp., *Dexiostoma* sp., *Euglena gracilis*, *Euplotes aediculatus*, *Paramecium aurelia*, *P. bursaria*, *Spirostomum* sp., *Tetrahymena* sp. and the rotifer *Cephalodella* sp. Of these species, *Chilomonas* sp., *Cyclidium* sp., *Dexiostoma* sp. and *Tetrahymena* sp. were supplied by Carolina Biological Supply Co., whereas all other species were originally isolated from a natural pond (McGrady-Steed, Harris & Morin 1997). The species were grown in sterilized culture medium made of local spring water, and $0 \cdot 45$ g L^{-1} of Protozoan Pellets (Carolina Biological Supply, NC USA). Protozoan Pellets provide nutrients for added bacteria (*Brevibacillus brevis*, *Bacillus subtilis* and *Serratia fonticola*). The experiments were conducted in a climatized room at 20°C under constant fluorescent light. Local communities were kept in culture well-plates containing 10 mL of culture medium.

THE INTERACTION EXPERIMENTS

All possible 55 pairwise species combinations were measured in replicated microcosms experiments. All 11 species were initially grown in pure cultures. Then, 5 mL of medium of species *i* at measured density ϕ_i^0 was mixed to 5 mL of species *j* at ϕ_j^0 (total volume, $V = 10$ mL). We furthermore tested the species' ability to coexist in communities composed of all 11 protists, initializing the microcosms by taking $V/11$ medium volume from each species' pure culture. The 11 1-species, the 55 2-species and the 11-species communities were replicated six times each. After three weeks, at $t^\star = 21$ days, we measured the density of each species in all microcosms. We sampled a variable quantity of medium and counted densities under a stereo-microscope, using previously established protocols (Altermatt *et al.* 2011; Altermatt, Schreiber & Holyoak 2011). The density of *Spirostomum* sp. was directly counted in the well-plates, as it naturally occurs at low densities.

MEASURING SPECIES INTERACTIONS

We used four methods to analyse the outcome of the interaction experiment ('Extinction' = *EX*, 'Relative Yield' = *RY*, 'LV Equilibrium' = *EQ*, 'LV Dynamics' = *LVD*; see Table 1 for a synthesis of the methods). The first three methods usually require a long-term experiment. The *LVD* method, instead, can be adopted in shorter experiments, but requires more information compared to the others (e.g. information on species characteristics such as intrinsic growth rates and carrying capacities is needed).

Interaction strengths

The change in the population density of species *i* over time due to the presence of species $1, \ldots, S$ can be written as

$$\frac{d\phi_i}{dt} = \phi_i f_i (\phi_1, \ldots, \phi_S), \qquad \text{eqn 1}$$

where f_i is the per-capita growth rate of species *i*. The interaction coefficients are mathematically described as the change in the per-capita growth rate of species *i* under a small change in density of species *j*

$$\alpha_{ij} = \frac{\partial f_i(\phi_i, \ldots, \phi_S)}{\partial \phi_j}. \qquad \text{eqn 2}$$

In all the four methods, the parameter α_{ij} reveals the strength of interaction of species *j* on species *i*. The absolute values of the αs are not directly comparable among the four different methods, because they are derived from different mathematical formulas (Table 1). For a given community, the interaction matrix summarizes all α-values (for data, see Carrara *et al.* 2015b). In every method but *EX* (see methods' details below), each α-value is the average over the six replicates. Most often, the quantity α_{ij} cannot be computed directly from experimental data. Each method discussed in the following approximates α_{ij} with ad hoc formula based on the available data.

Extinction. The *EX* method is counting the number of extinctions of species *i* caused by species *j*, across all the six replicates: $\alpha_{ij}^{EX} = \sum_{\text{rep}=1}^{6} \left(\delta_{\phi_{i|j,0}^\star} \right)$, where δ is the Kronecker's delta, $\phi_{i|j}^\star$ is the density of individuals of species *i* interacting with individuals of species *j*, at the sampling time $t^\star = 21$ days, and index *rep* sums over the six experimental replicates.

RELATIVE YIELD

The *RY* method compares the reduction (or increase) in population density of species *i* caused by species *j* relative to the 1-species performance of *i* (Loreau & Hector 2001), $\alpha_{ij}^{RY} = \phi_{i|j}^\star / K_i - 1$. With this method, $\alpha_{ij}^{RY} \geq -1$ by definition, with $\alpha_{ij}^{RY} = -1$ when species *j* has competitively excluded species *i*. This approach is in close analogy to the Paine index $PI = (\phi_i - K_i)/K_i\phi_j$ (Paine 1992), but it is not scaled for the abundance of the interacting species.

Lotka-Volterra equilibrium

Both methods *EQ* and *LVD* are based on a Lotka-Volterra (LV) model, where *EQ* assumes equilibrium conditions at sampling time, while the *LVD* does not. In LV models, the dynamics of species *i* and species *j* are characterized by the following phenomenological equation

$$\frac{d\phi_i}{dt} = r_i\phi_i \left(1 + \frac{\alpha_{ii}\phi_i + \alpha_{ij}\phi_j}{K_i} \right), \qquad \text{eqn 3}$$

where α_{ij} measures the strength of interspecific competition and α_{ii} that of intraspecific competition, which is equal to -1 for all species. Method *EQ* makes the crucial assumption that all communities have reached their equilibrium point at the time of measure. To extract the interaction coefficients by this method, species' intrinsic growth rates and initial conditions play no role in the characterization of species interactions. The only important component is the imbalance of the species' density in isolation compared to its density in the presence of another species. In principle, *EQ* does not explicitly require species

intrinsic growth rates, even though they are needed to test the equilibrium hypothesis (Dormann & Roxburgh 2005). In our study, we estimated the time for the attainment of equilibrium as 3 weeks (covering up to dozens of generations), also based on previous experiments made in similar set-ups (Cadotte *et al.* 2006; Haddad *et al.* 2008), where this method was used to quantify interaction strengths. Interspecific interaction strengths are measured by setting the temporal derivative in eqn (3) equal to zero (equilibrium condition), and solving for the interaction term $\alpha_{ij}^{EQ} = (\phi_i^\star - K_i)/\phi_j^\star$, where $\phi_i^\star$ and $\phi_j^\star$ represent densities for species i and j at time $t^\star = 21$ days. After rescaling the density of species i by its carrying capacity K_i, $n_i = \phi_i/K_i$, $\alpha'_{ij} = \alpha_{ij}K_j/K_i$ (Kokkoris *et al.* 2002), the LV model becomes

$$\frac{dn_i}{dt} = r_i n_i \left(1 - n_i + \alpha'_{ij} n_j \right). \qquad \text{eqn 4}$$

We rescaled the interaction coefficients in order to compare the effective per-capita interaction values in a multispecies context once being discounted by the values of the carrying capacities. In ecological systems composed of two species occurring at similar densities, such a rescaling would not be strictly necessary, as a relationship of the type $(\phi_i - K_i)/\phi_j$ is obtained solving for the equilibrium eqn (3). However, the rescaling is needed to achieve a more intuitive comparison of the interaction coefficients, especially when the densities among species span several orders of magnitude in absolute numbers. This is especially the case in a multispecies scenario (see paragraph *Community model* below), and in such it is therefore common practice to rescale the interaction matrix (see, e.g., Kokkoris *et al.* 2002). In our case, for example, the carrying capacity of *Euglena gracilis* is of the order of $K \approx 10^5$ ind mL^{-1}, whereas that of *Spirostomum* sp. is $K \approx 10^1$ ind mL^{-1}. Solving for the equilibrium eqn (4), the rescaled interaction coefficient $(\alpha_{ij}^{EQ})' = (n_i^\star - 1)/n_j^\star$ is obtained, where $n_i^\star$ and $n_j^\star$ represent now the rescaled densities for species i and j at time $t^\star = 21$ days. The values of α' were constrained into the domain $|\alpha'| < 5$, because this is limited by the experimental resolution (see Fig. S2). From this point on, unless otherwise specified, the rescaled quantities for the interaction coefficients will be given omitting the prime.

Lotka-Volterra dynamics. For the *LVD* method, interactions α_{ij}^{LVD} were derived without assuming equilibrium of the community at the final time point $t^\star$. The estimates of the interaction terms are obtained by fitting the time series of the 2-species interaction through a LV dynamical model, constrained to the initial conditions adopted in the experiment, n_i^0, n_j^0, and the final (rescaled) densities $n_i^\star, n_j^\star$ at $t^\star$. The fit of the trajectory was done by performing a least square minimization procedure, weighting the errors with the inverse of the species density. The fit was performed as a single time trajectory through the time points. The *LVD* method integrates the information of growth rate r_i and the carrying capacity K_i, which are obtained from the 1-species experiments.

INTERACTION TYPE

Species with competitive interactions have negative α-values $(-/-)$. The sign of α is positive when a predator–prey $(+/-)$ or mutualistic interaction $(+/+)$ is occurring between two species (although not in *EX*). A predator–prey interaction *i-j* has $\alpha_{ij}\alpha_{ji} < 0$. In mutualistic interactions, both α_{ij} and α_{ji} are positive ($\phi_i > K_i$, $\phi_j > K_j$). Amenalism/commensalism arises when one α-value is equal to zero and the other is negative/positive, respectively. Non-interacting (neutral) species have both α-values equal to zero (Fig. 1). We assigned the
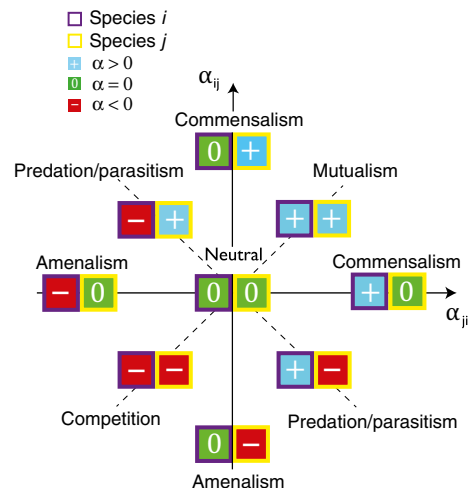

Fig. 1. Schematic illustration of all possible pairwise interactions between species i and j. The constants α_{ij} and α_{ji} describe the effect of species j on species i and the effect of species i on species j, respectively. The y- and x-axes are directly comparable to all subsequent interaction strengths α_{ij} and α_{ji} (see Fig. 3). For competitive interactions, both α-values are negative (red squares, loss–loss relationship), whereas in mutualistic interactions, both α-values are positive (cyan squares, win–win relationship). Predation/parasitism occurs where the two interactions have opposite signs ($\alpha_{ij}\alpha_{ji} < 0$). Non-interacting species fall on the origin of the graph.

interaction type and α-values for each species pair using the above-described categories, by considering the experimental uncertainty associated with each α-value as the confidence interval. Uncertainties on α-values were obtained by propagating errors from equations in Table 1 (for method *EX* by bootstrapping over the six replicates), thus taking into account the natural variation associated with the value of K-values, measured in the isolation experiment from six replicates. Differences in intra- vs. inter-group distributions of species interaction strength were tested with a Kolmogorov–Smirnov test on the cumulative distributions.

RANKING

We predicted the performance, in terms of final biomass production, for each of the eleven species in the 11-species experiments through a ranking procedure from the pairwise interactions. Species were ranked by their competitive ability, which was derived by subtracting the competitive effects of the responses of a species on all other species in the community in the pairwise interaction rounds (Roxburgh & Wilson 2000; Mouquet *et al.* 2004; Haddad *et al.* 2008). We obtained a species ranking measure (R) from the interaction matrix, by summing the values of the columns (community responses) and subtracting the values of the rows (community effects, Miller & Werner 1987), and then taking the absolute value. For species i, this is $R_i = \left| \sum_j (\alpha_{ij} - \alpha_{ji}) \right|$. Rescaling R between 1 and 11 allowed to make statistical comparisons between the four methods. As we are not expecting a precise relationship, statistical significance (weighted least square method with experimental errors) between R and normalized species density n_i was tested with power laws, varying the exponents between one (linear relationship) and five (highly nonlinear relationship), and with an exponential relationship.

COMMUNITY MODEL

Generalizing eqn (3) to a community with $S = 11$ species, a system of coupled differential equations is derived (Vandermeer 1969; Kokkoris *et al.* 2002), where density changes of species i are described by

$$\frac{d\phi_i}{dt} = r_i\phi_i \left(1 - \frac{\phi_i - \sum_{j\neq i}\alpha_{ij}\phi_j}{K_i} \right), \qquad \text{eqn 5}$$

which after rescaling becomes

$$\frac{d\mathbf{n}}{dt} = \mathbf{rn}\,(1 + \mathbf{A'n}), \qquad \text{eqn 6}$$

where $\mathbf{A'}$ is the experimental interaction matrix rescaled to each species carrying capacity. We investigated community dynamics through simulations by using the experimental interaction matrix $\mathbf{A'}$ derived through *LVD* method. The system in eqn 6 is solved using an implicit Runge-Kutta scheme. All species are present with a known initial density of $\phi_s^0/11$, as in the main experiment.

Stochastic community model

Deterministic solutions are good approximations for species with high population densities and for large volume V of medium. In some cases, when the species' carrying capacity or the volume/area of the system may be small (less than hundred individuals), fluctuations around the macroscopic solutions may not be negligible. Thereby, a stochastic approach is essential (McKane & Newman 2005; Melbourne & Hastings 2008). By simulating the deterministic system of eqn (6) through the Gillespie algorithm (Gillespie 1977), we directly solved the master equation associated and thus employed demographic stochasticity (van Kampen 2007, see Supporting Information Appendix S2 for a detailed description of the stochastic implementation of the model). We also performed stochastic simulations where we added, in addition to the demographic noise, the experimental uncertainties on the α-values of the derived interaction matrix.

STABILITY ANALYSIS

By applying asymptotic stability theory, the reaction of an ecological system to a small perturbation from the equilibrium state is fully described by the eigenvalues of its community matrix (May 1972; Alle-

sina & Tang 2012). For the LV model, it coincides with the Jacobian matrix system, linearized at equilibrium $\mathbf{n}^\star = (n_1^\star, \ldots, n_S^\star)$, where we are assuming constant interaction coefficients (Berlow *et al.* 2004)

$$J = [\partial(dn_i/dt)\partial n_j]_{n^\star}. \qquad \text{eqn 7}$$

Thus, inserting eqn (6) in eqn (7), we obtain

$$J = \text{diag}\,(r_1 n_1^\star, \ldots, r_S n_S^\star)\,\mathbf{A'}, \qquad \text{eqn 8}$$

where diag(...) represents a diagonal matrix by listing its diagonal elements $\mathbf{rn}^\star$ at equilibrium. The community matrix predicts if the community will sustain the current biodiversity level, or if instead the system is in an unstable configuration, and will be more prone to rearrangements under environmental/community fluctuations (May 1972; Ives, Gross & Klug 1999). An equilibrium is stable if the real part of the dominant eigenvalue of J is negative, $\text{Re}(\lambda(J)) < 0$. We derived a (deterministic) asymptotic stability of our 11-species community by applying the above procedure, for both initial and final communities, by adopting the interaction matrix $\mathbf{A}$ derived from the *LVD* method. To test the significance of the deterministic stability analysis, we performed a stability analysis by considering the experimental variation on the intrinsic growth rate, the carrying capacities and the interaction matrix α-values. We ran simulations randomly extracting values from the interval $[\overline{x} \pm \sigma_x]$, where $\overline{x}$ is the mean value for the quantities x used in the derivation of the deterministic stability (x: r, K and α-values) and σ_x quantifies experimental uncertainty for x.

Results

Competitive exclusion was observed in many species combinations (207 population extinctions over 660 populations in total of 330 microcosms, Fig. 2a). The majority of observed interactions were of competitive nature (*RY* 67%, *EQ* 73%, *LVD* 56%). Furthermore, a consistent part were predator–prey interactions (*RY* 22%, *EQ* 11%, *LVD* 26%) and amenalistic interactions (*RY* 16%, *EQ* 16%, *LVD* 18%). No neutral, commensalistic or mutualistic interactions were observed (Figs 3 a–d, S3, S4a). Interactions of species belonging to different functional groups were distinct for both the nature (i.e. the signs in the α-pair, discriminating between competitive, predator/prey, amenalistic, commensalistic, mutualistic or neutral
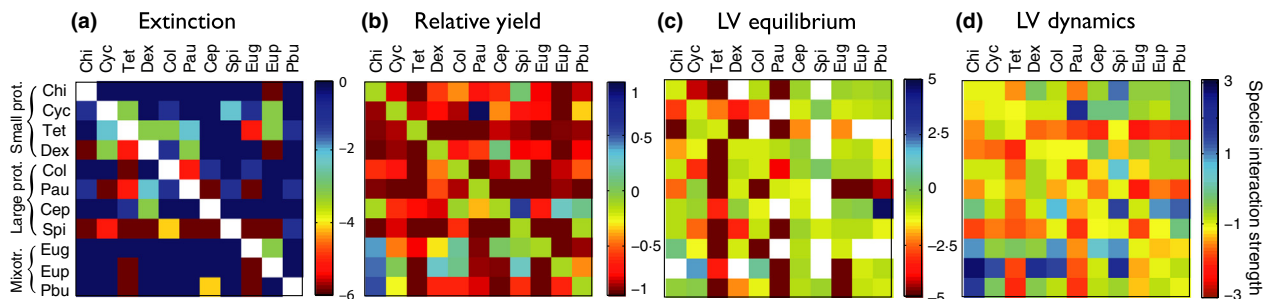

Fig. 2. Interaction matrices describing all experimentally measured pairwise interaction strengths between the 11 species obtained by four methods: (a) extinction method (*EX*), indicates the number of extinctions over the six experimental replicates; (b) relative yield method (*RY*), based on population reduction/increase, scaled to carrying capacity obtained in isolation; (c) Lotka-Volterra equilibrium method (*EQ*); and (d) Lotka-Volterra dynamics method (*LVD*). The colour of the square at position (*i,j*) indicates the effect of species *j* on species *i*. Colour bar indicates the strength and the sign of the interaction (red versus blue gradient, see Fig. 1). The absolute values of αs are not directly comparable. A blank square indicates a species' combination for which the inference of the interaction term was not applicable. The 11 species are as follows: *Chilomonas* sp. (*Chi*), *Cyclidium* sp. (*Cyc*), *Tetrahymena* sp. (*Tet*), *Dexiostoma* sp. (*Dex*), *Colpidium* sp. (*Col*), *Paramecium aurelia* (*Pau*), *Cephalodella* sp. (*Cep*), *Spirostomum* sp. (*Spi*), *Euglena gracilis* (*Eug*), *Euplotes aediculatus* (*Eup*) and *Paramecium bursaria* (*Pbu*). Species are ordered according to the three functional groups (small protists, large protists, mixotrophs) and with increasing body size within each group.
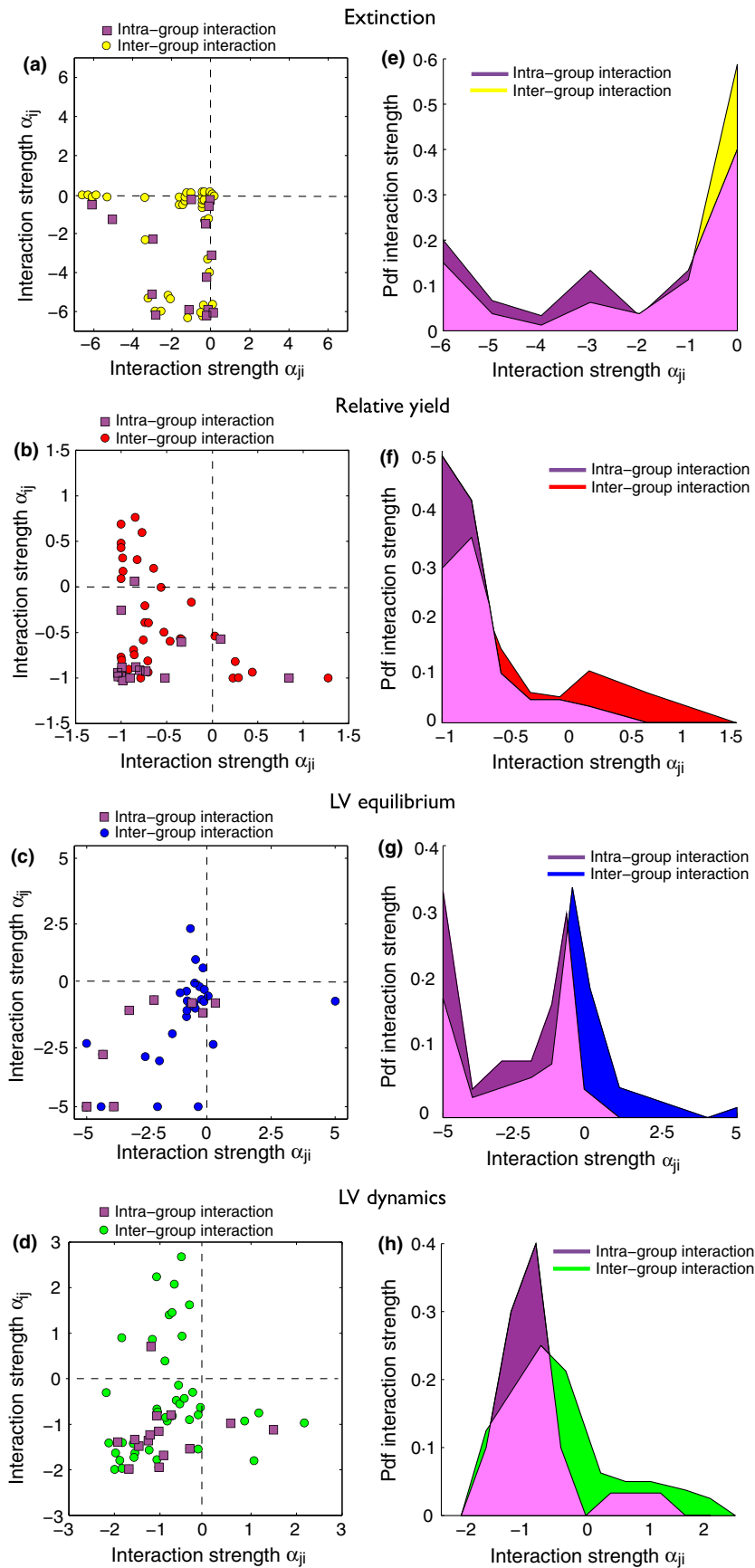
Fig. 3. (a–d) Distribution of interaction coefficients (α_{ij} vs α_{ji}) for all species combinations, measured by the four methods. (a) extinction (*EX*, yellow); (b) relative yield (*RY*, red); (c) Lotka-Volterra equilibrium (*EQ*, blue); and (d) Lotka-Volterra dynamics (*LVD*, green). The *RY* method has an upper boundary at $\alpha = -1$ by definition (competitive exclusion), whereas LV methods are constrained in $[-5,5]$ (see Fig. S2). Mutualistic links (both α-values > 0, see Fig. 1) in pairwise experiments of 11 protist species are missing in all methods. In magenta, species interactions from inter-functional groups are plotted. (e,h) Probability density function (*pdf*) of intra-group (magenta) and inter-group interaction strengths by the four methods. Lighter colour indicates where distributions overlap. (e) Intra-group interactions cause higher extinction rates and higher competitive strengths (left side in panels f–h).

interaction) and strength of the links (i.e. the absolute value of α-values in the interaction matrix). For intra-group interactions, competitive links were the majority (*RY* 80%, *EQ* 89%, *LVD* 80%), with very few predator–prey links (always less than 10%). For inter-group interactions, stronger predator–prey dynamics were detected (*RY* 27·5%, *EQ* 11%, *LVD* 32·5%), balanced by a lower proportion of competitive links (always less than 70%) compared to the intra-group interactions (Fig. S4b). Interaction strength distributions of intra- vs. inter-group interactions were significantly different in all but the *EX* method (Kolmogorov–Smirnov test, $P_{EX} = 0.39$; $P_{RY} = 0.05$; $P_{EQ} = 0.05$; $P_{LVD} = 0.005$; Fig. 3e–h).

Species competitive ranking, *R*, based on the pairwise interactions was a good predictor of species performance in terms of final biomass production in 11-species communities (normalized to each species' carrying capacity). The observed relationship between the rank and species performance is highly nonlinear (Fig. 4). *RY* was the best method in predicting biomass production from the pairwise interactions to the 11-species community (Fig. S5). *RY* was superior to the other methods irrespective of the relationship assumed in the fitting procedure (best fit for $n^\star/K = cR^4$). The worst predictor was the LV model based on equilibrium assumption ($r^2_{EX} = 0.81$, $r^2_{RY} = 0.88$, $r^2_{EQ} = 0.10$, $r^2_{LVD} = 0.79$). Only for the *EQ* method, the fit was not significant ($P_{EQ} = 0.41$). In fact, *EQ* poorly correlated with *RY* ($R = 0.41$, $P = 0.21$) and *LVD* ($R = 0.46$, $P = 0.16$), while it correlated with *EX* ($R = 0.74$, $P = 0.01$). A high degree of correlation was found between *RY* and *LVD* ($R = 0.96$, $P < 10^{-4}$, Fig. 5). *Spirostomum* sp. went extinct in all six replicates in our experimental conditions, but the results on the ranking were not affected by the removal of this species (Fig. S6).

Experimentally observed species richness of the 11-species communities was $\langle \alpha_{11} \rangle_{exp} = 7.5 \pm 0.55$ (mean ± SD). Numeri-
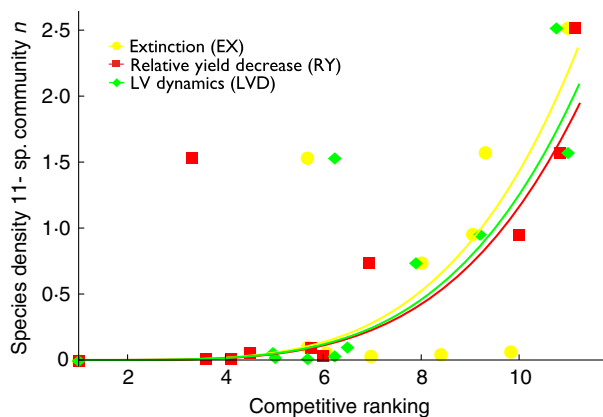

Fig. 4. Predicted species performance in 11-species community, based on the competitive rank *R* (rescaled between 1 and 11 for statistical comparison) obtained through different methods from the pairwise interaction experiment (extinction, yellow circles; relative yield, red squares; LV dynamics, green diamonds). The vertical axis gives the average species biomass normalized to each species' carrying capacity, $n = \phi/K$. Lines represent the best fit ($n \propto R^\delta$) with $\delta = 4$. *RY* is the best method in predicting species performances, for values of $\delta \in (1, 5)$ (similar results for fitting with an exponential curve). *EQ* has never provided significant results (Fig. S5).

cal deterministic simulations for the 11-species communities with the same experimental initial conditions, fitted to the pairwise experimental interaction strengths, showed the coexistence of six species (in these simulations, virtual species reflecting *Tetrahymena* sp., *Dexiostoma* sp., *Colpidium* sp., *P. aurelia* and *Spirostomum* sp. went extinct in all the 11-species simulations rounds, Fig. 6). Stochastic simulations implementing demographic stochasticity showed the stable coexistence with a maximum of eight species. Demographic noise was hampering coexistence: in the stochastic simulations, the average species richness was $\langle \alpha_{11} \rangle_{theo} = 5.2 \pm 1.06$.

Asymptotic stability analysis, using the (deterministic) community matrix derived with the *LVD* method, predicted the 11 species to be unstable both at the start and at the end of the experiment: $Re(\lambda(J[n_1^0, \ldots, n_S^0])) > 0$, $Re(\lambda(J[n_1^\star, \ldots, n_S^\star])) > 0$ (Fig. 7a). By considering the experimental uncertainties over the species' intrinsic growth rates, carrying capacities and α-values in the interaction matrix, the instability value detected from the deterministic analysis was not significantly different from the stability–instability boundary, that is the zero value for the real part of the dominant eigenvalue (Fig. 7).

Discussion

Different methodological and conceptual approaches have been used to measure species interactions in community ecology over the last decades (see, e.g., Paine 1992; Laska & Wootton 1998; Berlow *et al.* 2004; Dormann & Roxburgh 2005; Cadotte *et al.* 2006; Perkins, Holmes & Weltzin 2007; Haddad *et al.* 2008; Levine & Hille Ris Lambers 2009), hindering generalisations across studies. Furthermore, species interactions are often studied in a competition framework only, even though many other kinds of species interactions exist. To generally understand species interactions in functionally diverse communities and to extrapolate ecosystem processes of communities from pairwise interactions, an integrative and standardized approach is thus needed. Our study gives experimental results on consistently measured species interactions across different functional groups, suggesting a methodological framework for when and how to use different measures of species interactions.

SPECIES INTERACTIONS AND FUNCTIONAL GROUPS

We measured all pairwise interaction strengths among 11 protist species belonging to three functional groups by four methods (Fig. 2), implementing an increasing degree of model complexity (Table 1): 'Extinction' = *EX*, 'Relative Yield' = *RY*, 'LV Equilibrium' = *EQ* and 'LV Dynamics' = *LVD*. A consistent picture emerges from our analysis that spanned over different levels of resolution, focusing on extinction rates, biomass production or per-capita interaction rates (Figs 2, 3, S3, S4). Specifically, we observed that the proportion and the strength of competitive interactions for species of the same functional group was higher compared to inter-group interactions. This not only supports results from recent laboratory experiments which used microbial communities
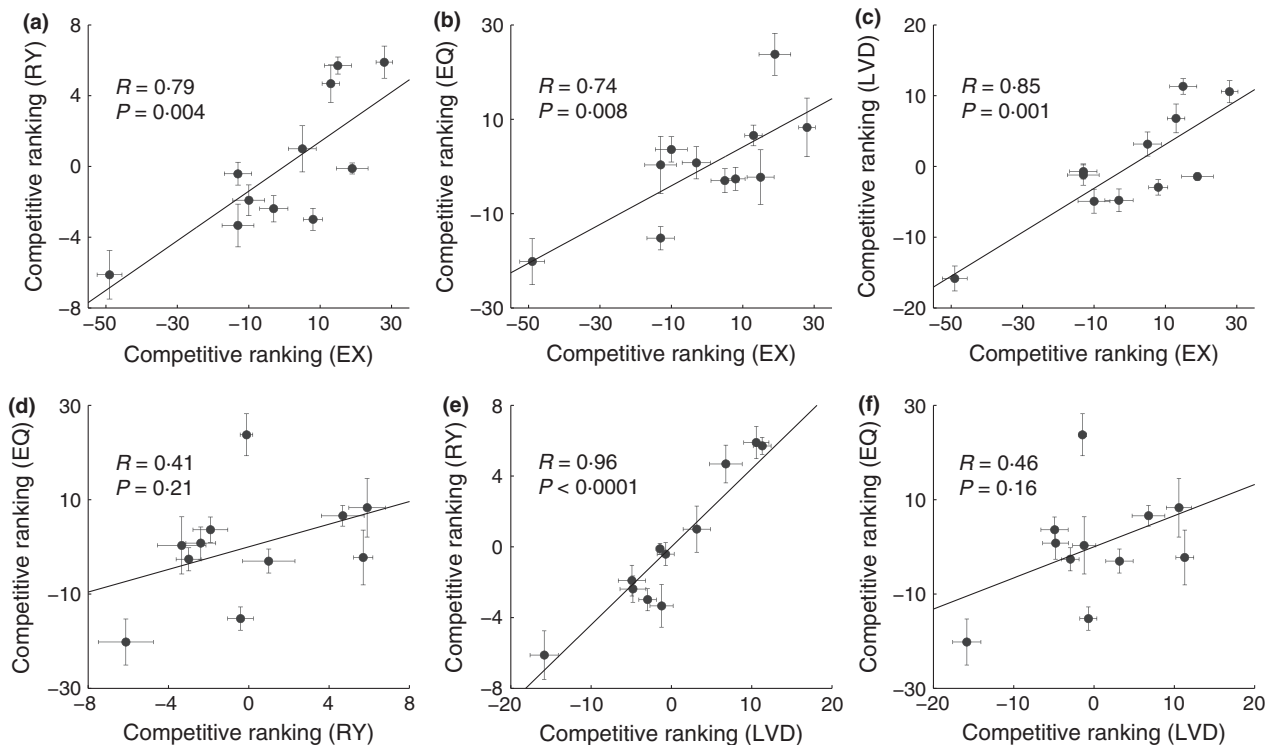
Fig. 5. Comparison among the four methods, extinction (*EX*), relative yield (*RY*), LV equilibrium (*EQ*) and LV dynamics (*LVD*), employed to estimate species rank, based on pairwise species interactions. Error bars represent the s.e.m. over the six experimental replicates.

with a simple trophic structure (Jiang, Tan & Pu 2010; Violle *et al.* 2011; Peay, Belisle & Fukami 2012; Tan *et al.* 2012), but also suggests that mechanisms of niche partitioning were more likely to occur between species belonging to different functional groups (Finke & Snyder 2008; Levine & Hiller Ris Lambers 2009; Eisenhauer *et al.* 2013; Carrara *et al.* 2015a).

EXTINCTION- AND POPULATION-BASED METHODS

We derived a competitive hierarchy from the interaction matrices (Roxburgh & Wilson 2000; Mouquet *et al.* 2004; Cadotte *et al.* 2006; Haddad *et al.* 2008) for each of the methods and used it to predict species performance in the 11-species communities. Generally, the rank extracted from the population-based method (*RY*) was the best in predicting the biomass production from pairwise interactions to the 11-species community, and it was superior to the other methods irrespective of the relationship assumed in the fitting procedure. Methods *EX* and *RY* (especially *RY*) are well suited for analyses of simple population and community dynamics, and they better apply to transitive communities (Freckleton & Watkinson 2001; Perkins, Holmes & Weltzin 2007). However, they do suffer a lack of predictability, especially for out-of-equilibrium or transient dynamics. Making predictions from pairwise to multispecies community with *EX* and *RY* methods have strong limitations when they are applied to large organisms which have longer generation times, because such methods depend on the effective observation of an extinction event, or on longer relaxation time to the equilibrium state.

LIMITATIONS OF THE LOTKA-VOLTERRA EQUILIBRIUM METHOD

The worst predictor was the LV model *EQ* based on the equilibrium assumption (Fig. S5). In fact, two main issues arise when adopting the *EQ* method, assuming equilibrium: (i) if species *j* gets extinct in combination with species *i* ($\phi_j^\star = 0$), then it is not possible to reconstruct α_{ij}, because of zeros in the denominator (see Table 1, *EQ* method); (ii) the equilibrium assumption may not be truly fulfilled. Paradoxically, when few individuals of species *j* are still surviving in combination with species *i*, but are likely on a path towards extinction, this may lead to an overestimation of α_{ij}^{EQ} because it is a per-capita-based model. This makes the method strongly dependent on experimental sampling times. Despite all these limitations, the LV modelling, which assumes equilibrium, is widely adopted to infer species interactions (e.g. Dormann & Roxburgh 2005; Haddad *et al.* 2008). Our results thus suggest that the *EQ*, even though conceptually among the first methods to chose from, suffers from severe experimental constraints given the strict equilibrium assumption. We suggest that extracting the ranking to project from the pairwise experiments to the multispecies communities with a LV (per-capita) models may not be the optimal strategy. In fact, while the ranking is an additive measure, LV models show inherently nonlinear dynamics. We have provided evidence that this procedure can result in completely false predictions (Fig. S5c). Therefore, simpler (population-based) methods, like balancing extinctions (Cadotte *et al.* 2006) or looking at population reduction/increase in competi-
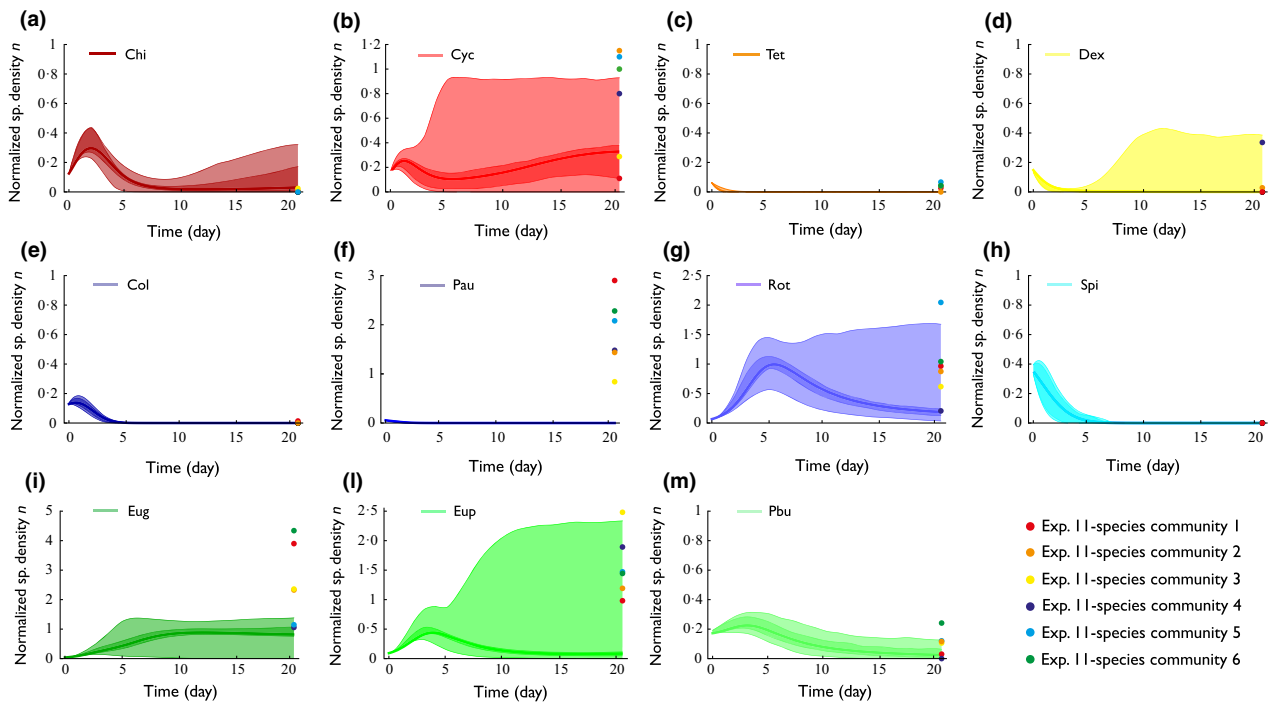
Fig. 6. Species temporal dynamics based on simulations adopting the community model fitted to the experimental pairwise interactions (*LVD* method, data rescaled to each species carrying capacity). Thick coloured lines are obtained by (deterministic) integration of the community model (eqn 6); darker coloured area (5th−95th percentile) refer to simulations employing demographic stochasticity; lighter coloured area (5th−95th percentile) refers to stochastic simulations, adding to the demographic noise the experimental uncertainties on the α-values of the derived interaction matrix. Coloured dots at $t = 21$ day refer to experimental species yields in the 11-species communities (each colour represents a different replicate). The systematic underestimation of *P. aurelia* (f) and *Euglena gracilis* (i) densities by our additive LV model suggests the emergence of positive non-additive effects (indirect forms of mutualism) in functionally diverse (and in trophically structured) microbial communities. These mechanisms may, for example, include the production of dissolved organic matter (DOM) and inorganic nutrients via sloppy feeding, and excretion by larger protists, available as food resource to *Euglena gracilis*. Furthermore, competition dynamics among bacteria and microflagellates in the lowest level of the trophic chain may have consequences for protists' dynamics.
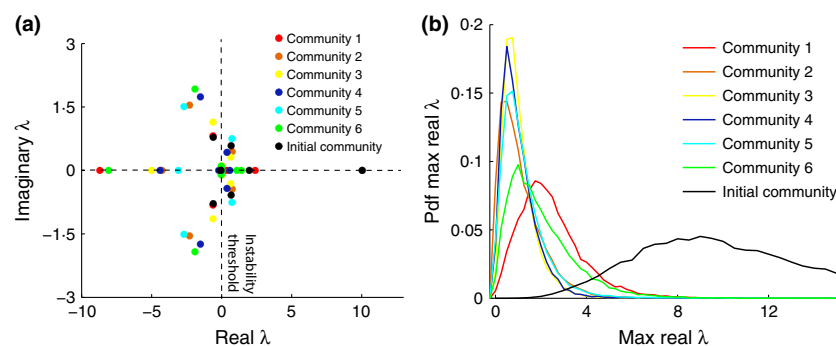

Fig. 7. (a) Asymptotic stability analysis of the Jacobian community matrix for the final configuration of all the six experimental replicates (coloured dots) of 11-species protist community (different colours for different experimental replicates) and for the initial community (black dots). On *xy*-axis real/imaginary parts of the eigenvalues are plotted. The deterministic analysis predicted the final configuration of the system to be in an unstable equilibrium, as highlighted by the positive real part of maximum eigenvalues in all the six community matrices. The initial configuration with each species s at its $K_s/11$ was highly unstable (black dot on the right side). (b) Probability density function of the maximum real part of community matrices eigenvalues, by considering experimental uncertainties on intrinsic growth rates r_s, carrying capacities K_s, and on the α-values of the interaction matrix, **A**. In four over six replicates, the distribution of eigenvalues is compatible ($P > 0.05$) with the zero value. Only the first (red line) and the sixth communities (green line) were predicted to be unstable. The stochastic stability analysis confirmed the instability for the initial community configuration (black line).

tion compared to the 1-species case (Paine 1992; Roxburgh & Wilson 2000), are better capturing each species' biomass production in multispecies communities at the final term of the experiment.

LOTKA-VOLTERRA DYNAMICS METHOD

The dynamical LV model *LVD* may capture the interaction coefficients from earlier dynamics, without the requirement of

reaching an equilibrium state, which is instead a firm assumption in the other three methods investigated here. Our stochastic implementation of the *LVD* model generally captures both the mean and the observed variability of species performances in multispecies communities (Fig. 6). Moreover, taking into account the experimental uncertainties in the species parameters, we showed how to complement a deterministic stability analysis with its stochastic counterpart, by deriving the distribution of the dominant eigenvalues of the Jacobian matrix (Fig. 7). However, the additive *LVD* model cannot directly capture non-additive effects (Case & Bender 1981), relative nonlinearities in intrinsic growth rate (Chesson 2000) or other forms of positive or negative interactions, such as interference competition (Amarasekare 2002) or prey switching (Glücksman *et al.* 2010), which may affect dynamics in natural communities, and likely occurred in our experimental communities (Fig. 6f,i).

Conclusions and recommendations

The main difference between methods *EX*, *RY*, *EQ* and *LVD* stands in the capability of the latter to generate dynamical predictions. This makes *EX*, *RY* and *EQ* suitable to test ecological theories – such as the limiting similarity hypothesis or in detecting niche partitioning mechanisms – but they cannot quantitatively predict community dynamics. The *EQ* method, based on Lotka-Volterra interactions with the equilibrium assumption, can give misleading results in predicting community properties from pairwise species interactions. With a detailed knowledge of the species' ecological traits, instead, a dynamical model such as the *LVD* is more accurate in capturing community dynamics.

When predicting the composition and stability properties of multispecies communities from pairwise competitive experiments, we strongly recommend to complement deterministic analyses with a stochastic approach, that is, taking into account the possible sources of variability (Ives, Gross & Klug 1999; de Mazancourt *et al.* 2013). Population and community dynamics incorporate nonlinear processes with inherent sources of stochasticity (McKane & Newman 2005; Melbourne & Hastings 2008). Thereby, a stochastic-based analysis results in a more accurate inference of population dynamics and community stability. Our stochastic implementation of method *LVD* is well suited to infer species' persistence times in interacting communities and to determine species' likelihoods of extinction. This has important implications for ecosystem management, in the selection of species combinations that are not only more productive in the short term, but also present higher stability over long time-scales.

Data accessibility

Descriptions of species and functional groups are uploaded as online Supporting Information. Details of community model uploaded as online supporting information. Interaction matrices for the four different methods (Carrara *et al.* 2015b).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Community dendrogram based on intrinsic growth rate, body size, and ability to photosynthesize as species traits.

Fig. S2. Simulated 2-species interaction dynamics adopting a Lotka-Volterra model, for (antisymmetric) values of $|\alpha_{ij}| = |\alpha_{ji}| = \alpha_{max} = 5$ and intrinsic growth rates $r_i = r_j = 1$/day (the average growth rate for the species in our communities).

Fig. S3. Interaction matrices describing all experimentally measured pairwise interaction strengths, averaged within each of the three functional groups (small protists, large protists, mixotrophs, see Fig. 2 for the species that are ordered in the same way) between the 11 species obtained by four methods: (a) extinction method (EX), indicates the number of extinctions over the six experimental replicates; (b) relative yield method (RY), based on population reduction/increase, scaled to carrying capacity obtained in isolation; (c) Lotka-Volterra equilibrium method (EQ), and (d) Lotka-Volterra dynamics method (LVD). The colour of the block at position (i, j) indicates the mean effect of species in functional group j on species in functional group i. Colour bar indicates the strength and the sign of the interaction (red versus blue gradient, see Fig. 1).

Fig. S4. (a) Relative proportion of competitive (red), predator-prey (brown), amenalistic (green), commensalistic (cyan), neutral (yellow),

and mutualistic (blue) interactions for all 11 species, by relative yield (RY), LV equilibrium (EQ), and LV dynamics (LVD) methods. (b) Relative proportion for intra- and inter-group interactions are given separately.

Fig. S5. Predicted species performance in 11-species community, based on the competitive rank R_i (rescaled between 1 and 11 for statistical comparison) obtained through different methods from the pairwise interaction experiment (a) extinction, circles; (b) relative yield, squares; (c) LV equilibrium, triangles; (d) LV dynamics, diamonds.

Fig. S6. Comparison between species ranks with and without *Spirostomum* sp., for (a) extinction (EX); (b) relative yield (RY); (c) LV equilibrium (EQ); (d) LV dynamics (LVD).

Appendix S1. Species traits and functional groups.

Appendix S2. Stochastic community model.