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Community ecology

Predicting biotic interactions and their variability in a changing environment

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Global environmental change is altering the patterns of biodiversity worldwide. Observation and theory suggest that species' distributions and abundances depend on a suite of processes, notably abiotic filtering and biotic interactions, both of which are constrained by species' phylogenetic history. Models predicting species distribution have historically mostly considered abiotic filtering and are only starting to integrate biotic interaction. However, using information on present interactions to forecast the future of biodiversity supposes that biotic interactions will not change when species are confronted with new environments. Using bacterial microcosms, we illustrate how biotic interactions can vary along an environmental gradient and how this variability can depend on the phylogenetic distance between interacting species.

1. Background

Global environmental change has substantial impacts on natural ecosystems [1,2]. Strong changes may doom species to extinction [1], relegate species to a smaller area of their original habitat [2,3] or may shift their distributions [4,5]. As a result, large-scale predictions about how species' distributions and abundances respond to the changing environment have become a priority [6,7] for both biodiversity conservation [8] and ecosystem management [9]. Species' distribution and abundance depend on multiple processes such as abiotic filtering [10–12], biotic interactions [3,5,13,14] and phylogenetic history [15]. Nevertheless, many species distribution models still rely on abiotic environmental variables (e.g. temperature [3]), effectively assuming that information on biotic interactions can be ignored [5,12]. Experimental evidence shows, however, that the assumption that biotic interactions are conserved along the abiotic environmental gradient is not necessarily true [16–18]. For instance, simulated warming using a community of fruit flies suggests that biotic interactions could change as we move along a temperature gradient, and that even subtle temperature change can alter the net effects of biotic interactions on species abundance [16,17].

Here, we test the joint effects of abiotic filtering and biotic interactions on the abundance of a bacterial species, and illustrate how these effects could depend on phylogenetic similarity of interacting species. We addressed this issue, using a model system where phylogenetically closer species have more similar abiotic environmental niches than distant ones [14], and hence where phylogenetic relatedness can inform on the patterns of biotic interactions among species in the assemblage [15].

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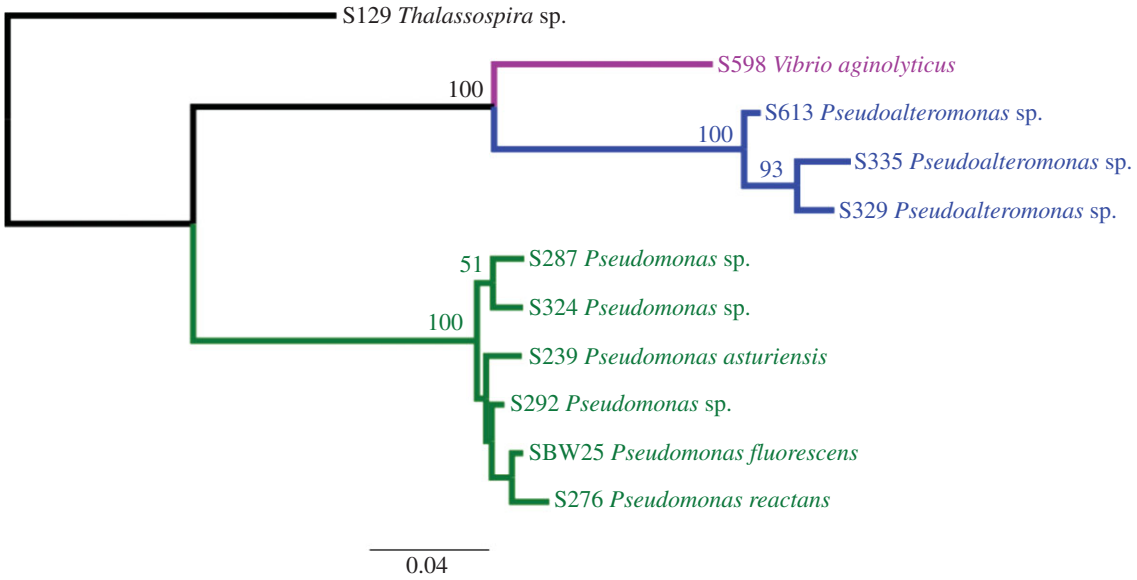

Figure 1. Phylogeny. Maximum-likelihood phylogenetic tree of the 11 marine strains. Numbers at nodes indicate maximum-likelihood bootstrap support values (more than 50). Phylogenetic distance was measured by the sum of branch lengths separating pairs of bacterial strains on the maximum-likelihood 16S rRNA phylogeny (patristic distances). (Online version in colour.)

2. Material and methods

We used bacterial communities composed of a focal species *Pseudomonas fluorescens* SBW25 (hereafter, SBW25), a well-characterized green fluorescent protein (GFP)-tagged model strain [19], and different target strains of freshwater and marine bacteria (electronic supplementary material, table S1). These target bacteria are a component of natural microbial assemblages near Montpellier, France [20], in environments in a gradient ranging from coastal rivers to saline lagoons. Given this natural gradient, we used salinity as a model abiotic filter [21]. We chose the target bacterial strains (electronic supplementary material, table S1) so that they had different optimal salinities for growth, and that phylogenetically close strains had more similar optimal salinity conditions than phylogenetically distant ones (i.e. phylogenetic similarity in species' abiotic environmental niche). We performed maximum-likelihood estimation of the phylogenetic tree of the 11 bacterial strains used (figure 1), and found that a strain's phylogenetic distance from the focal strain SBW25 is significantly correlated with a strain's optimal salt concentrations (Moran's $I = 0.452$, $p < 0.005$).

We used an 'experimental microbial biogeography' approach allowing for large-scale experimental design and substantial replication, where microplates are used to simulate the distributions of bacterial communities along a salinity gradient. We assembled mixtures consisting of SBW25 and another target strain on 48-well 1 ml microplates filled with LB medium supplemented with eight levels of salinity conditions in series (6.2–100 g l^{-1}, called 'cline' hereafter). After 48 h (a time period sufficient to cause competitive exclusion or dominance; electronic supplementary material, figure S1), we determined relative population densities of both marked SBW25 and its unmarked interactor in mixture (coculture experiment), using calibration techniques detailed in the electronic supplementary material, table S2 and figure S2. We also grew individual strains (the focal strain SBW25 and the 11 target strains in the electronic supplementary material, table S1) in isolation over 48 h along the salinity gradient. To infer the effects of biotic interactions, we contrasted the abundance of SBW25 in the co-culture and monoculture experiments. We calculated the proportional change (Δ) in population abundance of our focal strain SBW25 when grown alone versus in the presence of another strain: $\Delta = (D_{poly} - D_{mono})/D_{mono}$, where the abundance of SBW25 in monoculture is denoted by D_{mono} and that in co-culture by D_{poly}. The index Δ quantifies the effect of biotic interaction on population abundance of SBW25 at individual salinity levels, varying from -1 (exclusion from salinity levels where is otherwise suitable), through -0.5 (neutral partitioning), -0.5 to 0 (weak interaction), and higher (positive interactions). We analysed the effects of salinity and the identity of target strains on proportional change (Δ) in population abundance of SBW25 along a manipulated gradient of phylogenetic relatedness, using ANOVA and linear regression.

3. Results and discussion

We found that the average Δ value across all the co-culture treatments was -0.229 (± 0.231 s.d.). Out of 462 co-culture experiments (using the salinity range limited to 6.2–60 g l^{-1}, as none of the strains grew sufficiently over 100 g l^{-1}), 90.4% of the effects of target strain on the focal strain SBW25 were negative, among which 18% were of strong negative interaction ($\Delta \leq 0.5$). We observed positive interactions (e.g. facilitation) in 9.5% of treatments. These infrequent cases were mostly observed at higher salinity levels.

We found that the effect of a target strain on our focal strain SBW25 (i.e. Δ) depends on the level of salinity (table 1); the Δ decreased with increasing salinity ($p < 0.0001$) (figure 2*a*). Our data are thus inconsistent with the assumption that biotic interactions are constant along abiotic environmental gradients. The observed variability in the outcome of biotic interaction among the salinity levels reflects differences in tolerance of high salinity among strains, which helps cells to persist and gain relative fitness advantages from biotic interactions (electronic supplementary material, figures S3 and S4).

We assessed patterns in the variability of biotic interactions (i.e. Δ) along the salinity gradient and their relationship with phylogenetic relatedness of strains. To do this, we fitted a linear regression describing Δ as a function of salinity gradient for each replicate cline (salinity from 6.2 to 60 g l^{-1}); the slopes could vary from negative (stronger competition with increased salinity), through zero (Δ is constant) to positive (progressively weaker competition or facilitation). We found that the rate of

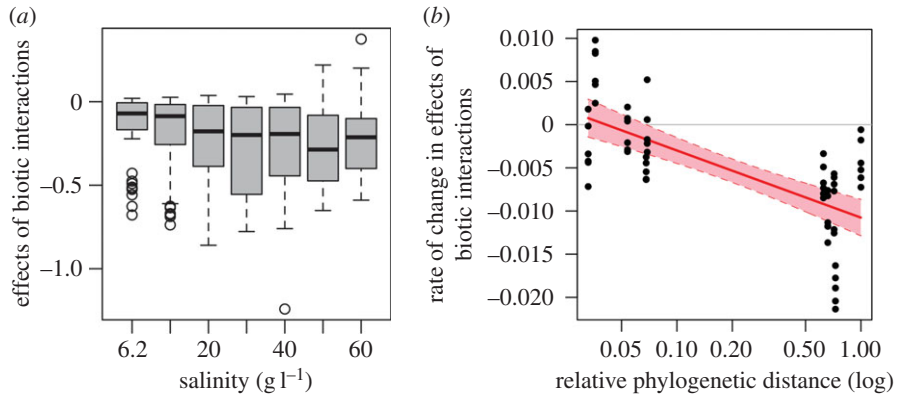

Figure 2. Effects of biotic interactions on species abundance of *Pseudomonas fluorescens* and their variability along salinity and phylogenetic gradients. (*a*) Effects of biotic interactions on species abundance of *Pseudomonas fluorescens* SBW25 becomes more negative with increasing salinity (data for all strain pairs included). At individual salinity levels, effects of biotic interactions (i.e. Δ) represent the proportional changes in population abundance of *P. fluorescens* SBW25 when grown alone versus in the presence of another competitor strain. Data points for 100 g l^{-1} salinity treatments were removed as neither strains grew well in the medium. (*b*) The more phylogenetically distant the strains in a community, the more the effects of biotic interactions change with elevated salinity. The predictor variable is phylogenetic distance between *Pseudomonas fluorescens* SBW25 and its paired target strain, with more negative being phylogenetically close to 1.00 being the most distant strain. The response variable is change in Δ along the salinity gradient for each replicate cline (i.e. the rate at which effects of biotic interactions on the abundance of the focal strain SBW25 change with elevated salinity). Data points for Wt (the wild-type of SBW25, hence phylogenetic distance is zero) were not shown, as log(relative phylogenetic distance) could not be calculated. (Online version in colour.)

Table 1. Results of ANOVA testing for the joint effects of salinity levels (6.2 – 60 g l^{-1}) and interacting target strain on the magnitude of biotic interactions Δ (quantified by the proportional change in population abundance of the focal strain SBW25 between monoculture and coculture treatments). The response variable Δ was square-root-transformed while retaining its sign to improve normality and homoscedasticity.

variable	d.f.	SS	MS	R^2	*F*	*p*-value
salinity level	6	1.371	0.2286	0.036	14.483	<0.0001
identity of interacting strain	10	21.751	2.1751	0.567	137.82	<0.0001
salinity level × identity of strain	60	9.145	0.1524	0.239	9.657	<0.0001
residual	385	6.076	0.0158	0.158		

change in Δ along the salinity gradient decreased markedly when paired with a phylogenetically distant strain (figure 2*b*; $t = -5.768$, $p < 0.001$). Despite the large variability in Δ within phylogenetically close or distant groups, the result indicates that phylogenetic relatedness predicts the variability of the rate of change in Δ among biotic interactions; the more phylogenetically distant the strains are in a community, the more likely the effects of biotic interactions would change with elevated salinity. For example, when SBW25 was mixed with the target strain S276, Δ remained more or less unchanged along the salinity gradient, suggesting that the effects of biotic interactions were constant at different salinity levels (electronic supplementary material, figure S5). By contrast, when the phylogenetically distant target strains S613 and S598 were used, Δ declined with elevated salinity (electronic supplementary material, figure S5). Mixtures of phylogenetically distant strains resulted in biotic interactions being more variable in a changing environment.

The results thus show that the effects of biotic interactions on species abundance are more likely to change for greater environmental change (figure 2*a*). This finding is consistent with theoretical work showing that competition can have more effect in extreme environments [13,22]. More work is needed to conclude on this last point however, as species' responses to deteriorating environmental conditions might also be asymmetric. Furthermore, our experimental design allowed us to link this variability in the effect of environmental change on biotic interactions to the phylogenetic distance between species (figure 2*b*). Even if the phylogenetic pattern was manipulated in our experiment, the results suggest that if biotic interactions are conserved in the phylogeny [14], closely related species could show less variability in their interactions when confronted with environmental changes than distant species.

Our work illustrates the potential for phylogeny to predict future effects of biotic interactions, but does not provide a mechanistic picture of these processes in natural ecosystems. By controlling phylogenetic community structure, we manipulated the response of the focal bacterial strain SBW25 to the salinity gradient and biotic interactions with other strains, but we also probably modified other variables such as the bacterial strain's foraging efficiency and chemical interactions. Indeed, multiple covarying abiotic and biotic factors are a feature of many prevalent properties of global environmental change [11,12]. Also, by choosing the bacterial strains such that phylogenetically close strains had more similar optimal salinity conditions than phylogenetically distant ones, we have created a 'model experiment' that does not allow generalization.

Despite these limitations, we believe our work will motivate ecologists to explore how the use of phylogeny can help to predict future biodiversity in a changing environment. It has

been stated that failure to incorporate biotic interaction into future species distribution models could produce misleading predictions [6,16]. But, if we are to use the present biotic interactions to forecast future species' distribution and abundance, then our results emphasize two fundamental limits. First, we cannot extrapolate too far along the anenvironmental gradient, because the biotic interactions can be very different in new environments. Second, biotic interactions with phylogenetically distant species might be harder to predict in new environments, because the magnitude of their interaction might be less conserved along an environmental gradient. Even if a general relationship between phylogenetic relatedness and niche similarity is not clear [14], unravelling these limits is an open challenge for ecologists that needs to be addressed to improve our ability to make predictions about future patterns of biodiversity in a changing world [7].

Data accessibility. The 16S rRNA sequences of the 12 bacterial strains have been deposited in the European Nucleotide Archive of EMBL-EBI under accession numbers LN868389–LN868398. http://www.ebi.ac.uk/ena/data/view/LN868389-LN868398. Raw data for the monoculture and polyculture experiments have been deposited in the Dryad: http://dx.doi.org/10.5061/dryad.v36v0.

Author's contributions. K.K. and N.M. conceived and designed the study, with suggestions and input from W.G. K.K. and C.G.B. performed the experiment, and K.K. analysed the data. F.D. performed phylogenetic analysis. K.K., N.M. and W.G. interpreted and discussed the results; K.K. wrote the first draft of the paper with substantial input from N.M. and W.G. All the authors contributed to revision. All the authors contributed substantially to conception and design, or acquisition of data, or analysis and interpretation of data, and revising the manuscript critically for important intellectual content. All the authors approved the final version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing interests. We have no competing interests.

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