ECOLOGY LETTERS

*Ecology Letters*, (2019) **22**: 1061–1071 doi: 10.1111/ele.13262

LETTER

# Warming reduces the effects of enrichment on stability and functioning across levels of organisation in an aquatic microbial ecosystem

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**Abstract**

Warming and nutrient enrichment are major environmental factors shaping ecological dynamics. However, cross-scale investigation of their combined effects by linking theory and experiments is lacking. We collected data from aquatic microbial ecosystems investigating the interactive effects of warming (constant and rising temperatures) and enrichment across levels of organisation and contrasted them with community models based on metabolic theory. We found high agreement between our observations and theoretical predictions: we observed in many cases the predicted antagonistic effects of high temperature and high enrichment across levels of organisation. Temporal stability of total biomass decreased with warming but did not differ across enrichment levels. Constant and rising temperature treatments with identical mean temperature did not show qualitative differences. Overall, we conclude that model and empirical results are in broad agreement due to robustness of the effects of temperature and enrichment, that the mitigating effects of temperature on effects of enrichment may be common, and that models based on metabolic theory provide qualitatively robust predictions of the combined ecological effects of enrichment and temperature.

**Keywords**

Body size, compositional resistance, interaction, microcosm, nutrient, temperature, temporal stability.

*Ecology Letters* (2019) **22**: 1061–1071

## INTRODUCTION

Temperature and resource enrichment are major drivers of global environmental change (Cross *et al.* 2015). Temperature directly affects vital rates of many organisms and indirectly affects population, community and ecosystem structure and dynamics. It controls the metabolic rate of cells (Gillooly *et al.* 2001) as well as their size [the temperature-size rule, Ohlberger (2013), Forster *et al.* (2012)], carbon allocation (García *et al.* 2018), population growth and carrying capacity (Fussmann *et al.* 2014; Plebani 2015), rates of biological interactions (Rall *et al.* 2012; Burnside *et al.* 2014; Fussmann *et al.* 2014) and ecosystem respiration (Yvon-Durocher *et al.* 2015). As a consequence of the interplay of many biological rates, warming is also expected to change the stability of ecological populations and communities (Fussmann *et al.* 2014; Uszko *et al.* 2017).

Resource enrichment, on the other hand, affects the material available for major biological processes such as growth, maintenance and reproduction in an organism (Sterner & Elser 2002). More nutrients usually result in larger individuals (Ohlberger 2013) and larger populations, though these effects are strongly mediated by the community in which species are embedded. Classic examples of such effects are the paradox of enrichment, where an increase in resource supply destabilises the population dynamics (Rosenzweig 1971). Furthermore, enrichment can affect the structure of food webs, for instance by determining the length of food chains (Oksanen *et al.* 1981; Kaunzinger & Morin 1998). Resource enrichment has therefore an important influence on the stability of communities and ecosystems.

While temperature and nutrient enrichment are well-studied in isolation, they often occur simultaneously (Cross *et al.* 2015, Fig. 1a). This allows for interactions, which can exacerbate (i.e. synergies) or mitigate (i.e. antagonisms) the effects of individual drivers (Brook *et al.* 2008), potentially limiting our ability to predict ecological dynamics (Garnier *et al.* 2017). Interactions can arise due to differential responses across levels of ecological organisation; hence studying how individual, population, community and ecosystem respond to temperature and enrichment in combination can provide a more integrative and complete understanding and help predict the joint impacts of global change drivers.

Previous studies analysing the joint effects of warming and enrichment rarely considered more than two levels of ecological organisation (Fig. 1b and Table S1). The majority of previous works studied effects on population and community biomass, whereas effects on ecosystem properties and individual-level information are usually not taken into account. Studies that have carried out analyses across multiple levels of organisation focusing on temperature concluded that the temperature-size rule is expected to maintain consumer-resource biomass ratios and buffer the community from extinctions under warming (DeLong *et al.* 2015; Osmond *et al.* 2017). However, how the combined effect of temperature and enrichment varies across levels organisation is lacking consensus due to insufficient empirical investigation (Cross *et al.* 2015). Hence, there is a knowledge gap about how changes in one level may influence dynamics of other levels and how this translates into ecosystem functioning and stability (Levin 1992). Due to the logistical challenges of studying dynamics

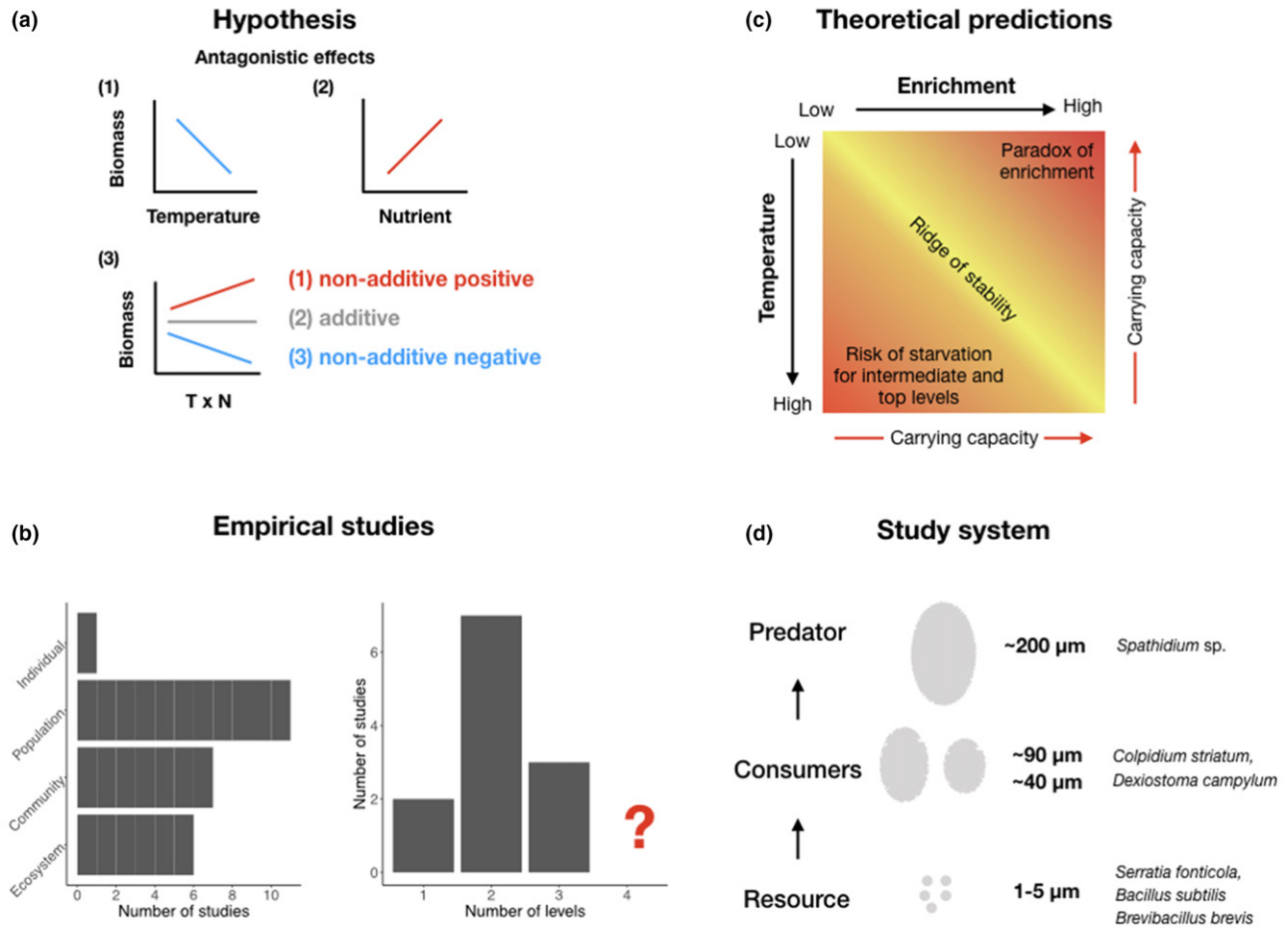


**Figure 1** Temperature and nutrient interaction. (a) Single effect of temperature and nutrient enrichment are well-studied, but their interaction varies in empirical studies. Here we hypothesise that temperature decreases and nutrient enrichment increases the biomass and their interaction can be (2) linear (additive) or (1) and (3) non-additive positive or negative. (b) Review of previous empirical work on temperature-nutrient interactions in aquatic microbial systems. (c) Conceptual summary of modelling assumptions and predictions by Binzer *et al.* (2012). Black arrows show the fertilisation and temperature gradients. Red arrows show the model assumptions regarding carrying capacity of the basal species with enrichment and temperature. Inside the plot the most important model predictions with the most structured body size distribution (consumers are 100 times larger than the basal species). At high fertilisation and low temperature, the system destabilises due to oscillations; at high temperature and low enrichment predators face starvation. Both ends of the spectrum are considered unstable states (stability here defined as not oscillating dynamics). (d) Our experimental study system with three trophic levels and their body size structure (cell length).

across levels of ecological organisation, much of our current understanding about joint effects of temperature and nutrients across levels of organisation is based on theoretical work.

Binzer *et al.* (2012) investigated the interactions between temperature and nutrient effects using a body size and temperature-dependent consumer-resource model to simulate a three level food chain (Fig. 1c). Vital rates in this model scale with temperature according to the Arrhenius equation or hump-shaped relationships. The model assumed that increased energy input linearly increased the carrying capacity of the basal trophic level, but does not affect the growth rate. Enrichment destabilised the system by shifting biomass up the trophic levels in the long-term causing oscillations driving the top and the intermediate level to extinction [paradox of enrichment (Rosenzweig 1971); the principle of energy flux (Rip & McCann 2011)]. Warming at a constant nutrient level decreased the carrying capacity and increased the metabolism of the intermediate and top species level stabilising biomass dynamics. At higher temperature, the top and intermediate species levels were prone to starvation due to lower ingestion efficiency (i.e. the ratio of ingestion and metabolism of a species). At higher temperature, the system can take up more nutrients before it starts oscillating, that is warming stabilises the system at high enrichment (Binzer *et al.* 2012). A recent extension of the model investigated how the temperature–size response of individuals can modulate the dynamics of the food chain (Sentis *et al.* 2017). The model showed that the direction and strength of the temperature–size response can change the persistence of the food web, with a more structured body size distribution, that is larger differences in predator–prey size ratios, in general leading to greater stability. So far, these predictions remain empirically untested.

Most climate change scenarios expect temperature to rise over the next 100 years, with average increases between 2 and 4 °C (IPCC 2007). In contrast, empirical and theoretical studies assess how temperature affects ecological systems by imposing a constant temperature increase rather than gradual increase. Whereas instantaneous exposure to increased

temperature represents a strong selection pressure to which organisms may be able to adapt, slowly increasing temperatures present a weaker selection pressure which may allow for easier acclimation and adaptation and hence may lead to differential effects on individuals and populations. Differential responses to gradual vs. instantaneous temperature increase have been shown to affect critical thermal limits such as the $CT_{max}$, a measure widely used in evolutionary biology to assess the potential for adaptation (Rezende *et al*. 2011). The ecological implications of constant vs. gradually increasing temperature have been insufficiently explored, despite potential for population-level responses trickling up to the community and ecosystem level (Fox & Morin 2001).

The aim of our study was to understand how warming and nutrient enrichment affect ecological dynamics and stability across levels of ecological organisation in both constant temperature and gradually warming environments. To do so, we conducted an experiment with an aquatic microbial community with three trophic levels. Protists have a long tradition as model organisms and have been used to investigate concepts in population and community ecology due to their fast generation time that allows to collect time series data on their dynamics under controlled experimental conditions (Altermatt *et al*. 2015). Furthermore, protist growth rates are strongly temperature-dependent (Fox & Morin 2001) which allows for investigating the long-term effects of different environmental manipulations on their ecological dynamics. We recorded time series of population biomass and cell size for each trophic level and measured community and ecosystem level variables.

Overall, we hypothesise that temperature and nutrient enrichment act in opposite directions, that is that they have antagonistic effects *sensu* Piggott *et al*. (2015) and act non-additively in combination across levels of organisation (Fig. 1a). We then qualitatively compared the experimental results to the following predictions of available theoretical studies (Binzer *et al*. 2012; Sentis *et al*. 2017): (1) temperature decreases and nutrient enrichment increases carrying capacity; (2) an interactive effect between high enrichment and high temperature on community biomass that counteracts the detrimental effects of warming; (3) a decrease in consumer temporal stability at high enrichment and low temperature. We also assessed if some of the mechanisms responsible for these patterns matched: (3.1) Nutrient-rich environment saves species from warming-induced starvation; (3.2) at high temperatures consumers in nutrient-poor communities run a risk of starvation because of a lower ratio of ingestion to metabolism; (4) individual cell size decreases with temperature, and the effect is exacerbated by low-resource availability. We also explored the potential importance of any differences between the assumptions of the model and the possible features of the experimental system.

## METHODS

### Community experiment

#### *Microbial food web*

We factorially manipulated temperature and nutrient availability to disentangle their effects in isolation and potential interactions across levels of ecological organisation in a simple heterotrophic microbial food web. The basal trophic level consisted of a mix of three bacteria species (*Bacillus subtilis*, *Serratia fonticola* and *Brevibacillus brevis*) decomposing the filtered organic medium (protist pellets, Carolina Biological Supplies, Burlington, NC in Chalkley's medium (Altermatt *et al*. 2015). Two bacteriovorus ciliates (*Colpidium striatum* and *Dexiostoma campylum*) constitute the intermediate consumers in the system feeding on bacteria. Both consumers are fed on by the top predator (*Spathidium* sp.), which cannot survive on bacterial prey (Fig. 1d). All populations were heterotrophic. Although we do not have direct evidence about genetic variation within populations, the culture conditions favour occasional sexual reproduction (i.e. triggered by resource depletion) and accumulation of mutations providing standing genetic variation. Each community was started by preparing the medium with the three nutrient levels. We then transferred bacteria into the medium using inoculation loops and incubated the cultures at 37 °C for 1 day, which gives them enough time to reach carrying capacity. We then added 300 individuals of each protist species (*Colpidium* and *Dexiostoma* to 100 mL medium with bacteria in previously autoclaved glass bottles (GL 45, Schott Duran, Germany). *Spathidium* was added to the cultures twice, to assure establishment in each microcosm. Ten individuals of the top predator were added 3 days after the introduction of the consumer species, and another 10 individuals after 6 days.

#### *Experimental design*

The temperature treatment had four levels: three constant temperature treatments of 15, 20 and 25 °C, and one rising temperature treatment with rate of + 2 °C per week starting at 15 °C and ending at 25 °C, hence with a mean temperature of 20 °C comparable to the constant treatment. These rates of increase match expected per generation rates of increase that larger organisms are predicted to experience over the next several decades (IPCC 2007). Furthermore, the temperature range is suitable for studying dynamical changes based on the thermal response of the selected species. Temperature was controlled with programmed incubators (Pol-Eko Aparatura, Wodzislaw, Poland), with two running each of the four temperature treatments. Based on previous experiments with the same laboratory strains, we know that both of the intermediate consumers show a slow decrease in carrying capacity when temperature is higher than 21 °C, but only *Colpidium* shows a decrease in growth rate with higher temperature (Jiang & Morin 2004; Plebani 2015). *Dexiostoma campylum* increases growth rate more than threefold between 10 and 20 °C (Laybourn-Parry 1984). The thermal niche of *Spathidium* sp. was pretested and showed viable populations across the temperature gradient. The predator increased its feeding rate and decreased its handling time with temperature, with the highest performance at 25 °C. Generation times for the two consumer species are on the scale of 2–3 generations per day under optimal growth conditions, whereas the predator generation time is about 0.5 per day. 5.5 weeks would mean about 100 generations for the consumers and 50 for the predator. Therefore, the duration of the experiment could allow for adaptation or acclimation. The three levels of protist pellet medium were used to create different nutrient levels: 0.275 mg (low),

0.55 mg (medium) and 1.1 mg (high) per litre. Nutrient variation hence covered fourfold variation comparable to previous experiments in which changes in nutrients led to changes in food chain properties (Kaunzinger & Morin 1998). Subsequent filtering with a mesh size of 0.45 μm removed large particles from the medium. Overall, the design yielded 12 treatment combinations, with six replicates, resulting in 72 microcosms studied (6 * 12 = 72 experimental communities).

*Sampling and time series of system dynamics*

Microcosms were sampled daily during the first week and then every third day until the end of the experiment after 38 days, to capture time series of the dynamical changes in body size, population size, community biomass and dissolved oxygen concentration. For each sampling, 5 mL medium (i.e. 5% of the total volume) was removed from each microcosm and replaced with 5 mL of sterile media (except for the first week, where 1 mL was sampled and replaced daily summing to a total of 5% total volume). This sample was subdivided to estimate bacterial biomass by flow cytometry, consumer abundance by video microscopy and predator abundance by manual microscopy.

The total number of bacteria was measured using flow cytometry (Accurri C6 with multi-well sampler, BD Biosciences, San Jose, CA, USA). We diluted a 20 μL sample tenfold by adding 160 μL filtered ionised water and SYBR green I (Invitrogen, Paisley, UK) solution in 96-well plates, resulting in a sample to SYBR green concentration of 1:1. Samples were then incubated at 37 °C for 15 min to stain the DNA in each cell. The multiwell plates were automatically measured, providing a cell count (abundance) and individual cell volumes for each bacterial cell (in a fixed amount of sample). We calculated bacterial biomass by summing the cell volume of all individual cells (for details see section 2.4 in the Supporting Information).

Consumer abundance was quantified with video-microscopy techniques (Pennekamp & Schtickzelle 2013; Pennekamp *et al.* 2015, 2017). For each sample, the microcosm vessel was gently agitated, and 700 μL subsample was mounted onto a glass slide and covered with a glass lid. Five-second videos (at 25 frames per second) were taken using magnification on stereomicroscope (Leica M205 C) mounted with a digital CMOS camera (Hamamatsu Orca C11440, Hamamatsu Photonics, Japan). Video analysis was used to count individuals and measure their morphology (i.e. cell size) and movement behaviour using the R package BEMOVI (version 1.0.2) (Pennekamp *et al.* 2015). Morphology and movement traits were used to classify individuals into the two consumer species using random forest classification (Pennekamp *et al.* 2017). Filtering removed spurious trajectories due to background motion. Cell counts were extracted for each time point and we calculated biomass for both species. Individual-level body size information was extracted from video analysis as the area (cross-section) of each individual (in $\mu m^2$). We calculated the intermediate consumers' body sizes by averaging the area of individuals over the first 10 days of the experiment. We constrained body size information to the first 10 days, so we could make a fair comparison across treatments (in some treatments after that period the number of individuals was strongly lowered which makes the estimation less precise). For further details of the video processing refer to the Supporting Information.

The abundance of the top predator *Spathidium* sp. was too low to be reliably counted by video microscopy. Therefore, we manually counted individuals and cysts in 1 mL samples using light microscopy. We also calculated biomass by multiplying the number of individuals with their average cell volume. Dissolved oxygen concentration (DO) (units of $\mu mol\ mL^{-1}$) was measured using a non-invasive method using a chemical-optical sensor (Fibox 4 trace, PreSens, Germany).

**Response variable and analyses**

We calculated six response variables: body size, biomass proportion, temporal stability of community biomass, compositional resistance, total biomass and the rate of biotic activity (see Table 1). Community biomass was the sum of all measured organisms' biomass. The rate of biotic activity quantifies how fast the dissolved oxygen concentration in the media changed. Organismal respiration contributes to reduction in dissolved oxygen while diffusion from the atmosphere contributes to increased dissolved oxygen. The more positive the rate, the higher the biotic activity is, with higher turnover in the system, i.e. more organisms (primarily bacteria) being consumed. The rate of biotic activity was estimated by fitting logistic curves to mass-corrected dissolved oxygen measurement in each microcosm (see further details in Supplementary Material).

To quantify stability, we calculated the inverse coefficient of variation as the measure of temporal stability (Lehman *et al.* 2000). A second stability component, compositional resistance, was estimated to describe changes in community composition and evenness (Baert *et al.* 2016). Compositional resistance was the Bray–Curtis dissimilarity of species biomasses between the low temperature – low enrichment treatment and each of the other treatment combinations. A compositional resistance of 1 indicates no effect of a treatment on composition, whereas 0 would indicate that the reference and a treatment have no species in common.

Our experimental design is a fully factorial manipulation of temperature and nutrient treatments under controlled conditions, therefore we used general linear models to test the treatment effects, that is the main and interaction effects of nutrient and temperature. Explanatory variables were the temperature treatment with four levels and the nutrient enrichment with three levels as well as their interactions. Analysis was separated where we used (1) only the constant temperature levels and where we used (2) the rising vs. 20 °C constant temperature levels. All models were tested for homogeneity of variances and normality in residuals.

To further examine changes in biomass within the community, we calculated the relative biomass of each population. Biomass measures were converted to the same unit ($\mu L\ mL^{-1}$ and divided by the total biomass), and the compositional change analysed with beta regression. Our experimental design tested a number of defined hypotheses and therefore we did not use model selection. All analyses were performed with R (R Core Team 2018).

**Table 1** Definition and calculation of response variables from individual to ecosystem level

| Variable | Description | Level | Unit |
|---|---|---|---|
| Body size | Cross section of individual body size (only available for intermediate consumers) | Individual | $\mu m^2$ |
| Biomass proportion | Relative biomasses of bacteria, intermediate consumers and the top predator | Population | % |
| Temporal stability | Inverse of the coefficient of variation (CV) of the total biomass | Community | – |
| Compositional resistance | Change in composition compared to the 15 degrees low nutrient treatment combination | Community | – |
| Total biomass | Sum of bacterial and protist biomasses (intermediate consumer species and top predator) | Community | μL/mL |
| The rate of biotic activity | The rate at which mass-corrected dissolved oxygen increases/decreases in the system | Ecosystem | $day^{-1}$ |

### Model robustness and putative empirical mechanisms

To test model robustness to parameter values and assumptions of the bioenergetic model (Binzer *et al*. 2012), we conducted a Global Sensitivity Analysis (GSA) with the R package FME (Soetaert & Petzoldt 2010). We tested the parameters sensitivity with the size structure of 0.01 g for basal species and 10 times larger mass for the consumers. This size structure reflects well the experimental food web. The GSA was conducted with 300 draws and all parameters were allowed to vary over 50% about their nominal value. The resulting effect on the mean of all state variables was estimated. We did not examine sensitivity of predictions to assumptions that would require structural changes to the model (e.g. same activation energy across species, type of temperature dependence of search rate). We furthermore examined whether the model assumption of increases in basal trophic level biomass with nutrient enrichment was met in the experimental system (see further details in Supplementary Material).

## RESULTS

### Ecosystem and community level effects of temperature and nutrient enrichment

Nutrient enrichment generally increased total community biomass, but less so at higher temperatures (Fig. 2a). Conversely, temperature strongly decreased community biomass in the high enrichment treatment but had no effect in low or medium enrichment treatments. The patterns represent a strong negative interactive effect of temperature and enrichment (Fig. 2e, Table S3). Community biomass and the effect of enrichment did not differ between the rising temperature and 20 °C constant treatment (Fig. 2f, Table S4).

The rate of biotic activity increased with temperature and also with enrichment and no interaction effects were detected (Fig. 2b). Overall the effects of enrichment and temperature on biotic activity were additive. The rate of biotic activity and the effect of enrichment did not differ between the rising temperature and 20 °C treatment.

Temporal stability of community biomass tended to decrease with temperature, though also exhibited considerable variability among replicates (Fig. 2c). There was no clear effect of enrichment and no apparent interactive effect of temperature and enrichment. Temporal stability and the effect of enrichment did not differ between the rising temperature and 20 °C treatment.

Temperature increased compositional resistance at high enrichment, but had no effect at medium enrichment, and decreased compositional resistance at low enrichment (Fig. 2d). Put another way, enrichment decreased compositional resistance at low and medium temperatures, but had much less effect at high temperature. These patterns represent a strong-positive interactive effect of temperature and enrichment. Compositional resistance and the effect of enrichment did not differ between the rising temperature and 20 °C treatment.

Overall, the mass-corrected rate of biotic activity generally increased across temperature despite constant, increasing or decreasing compositional similarity with temperature. In contrast, temporal stability consistently decreased across temperature hence showing the opposite trend than the rate of biotic activity due to the overall slower metabolism at lower temperatures.

We conducted an analysis on total community biomass time series and we found evidence of transient dynamics of treatment effects at the beginning of the experiment (within the first 10 days). After the transient phase, effect sizes were consistent through time, closely represented by the averaged effect size (Fig. S4).

### Changes in relative biomass among species

High nutrient enrichment caused an increase in relative biomass of the two consumer species and decreased the relative biomass of the bacteria and the predator (Fig. 3 and Fig. S2). These effects of enrichment were, however, often weaker at higher temperatures, representing a negative interaction between temperature and enrichment (Table S5). Temperature had different effects on the relative biomass of different species that often interacted with enrichment. For example, bacteria showed an antagonistic interaction effect in high temperature and high nutrient treatments. Enrichment had an overall negative effect on relative bacterial biomass, but temperature response was not unidirectional. In contrast, *Colpidium* exhibited negative effects of temperature on relative biomass, which were stronger at higher temperatures. This decline in relative biomass of one of the consumers with temperature could have driven the lower temporal stability of community biomass observed. Temperature did not affect the relative biomass between the rising temperature and 20 °C treatment; only nutrient enrichment increased the proportion of consumers and decreased the relative biomass of the resource (Table S6).

### Body size

Temperature tended to increase the size of *Colpidium* but decrease the size of *Dexiostoma* (Fig. 4a and b, Table S7).

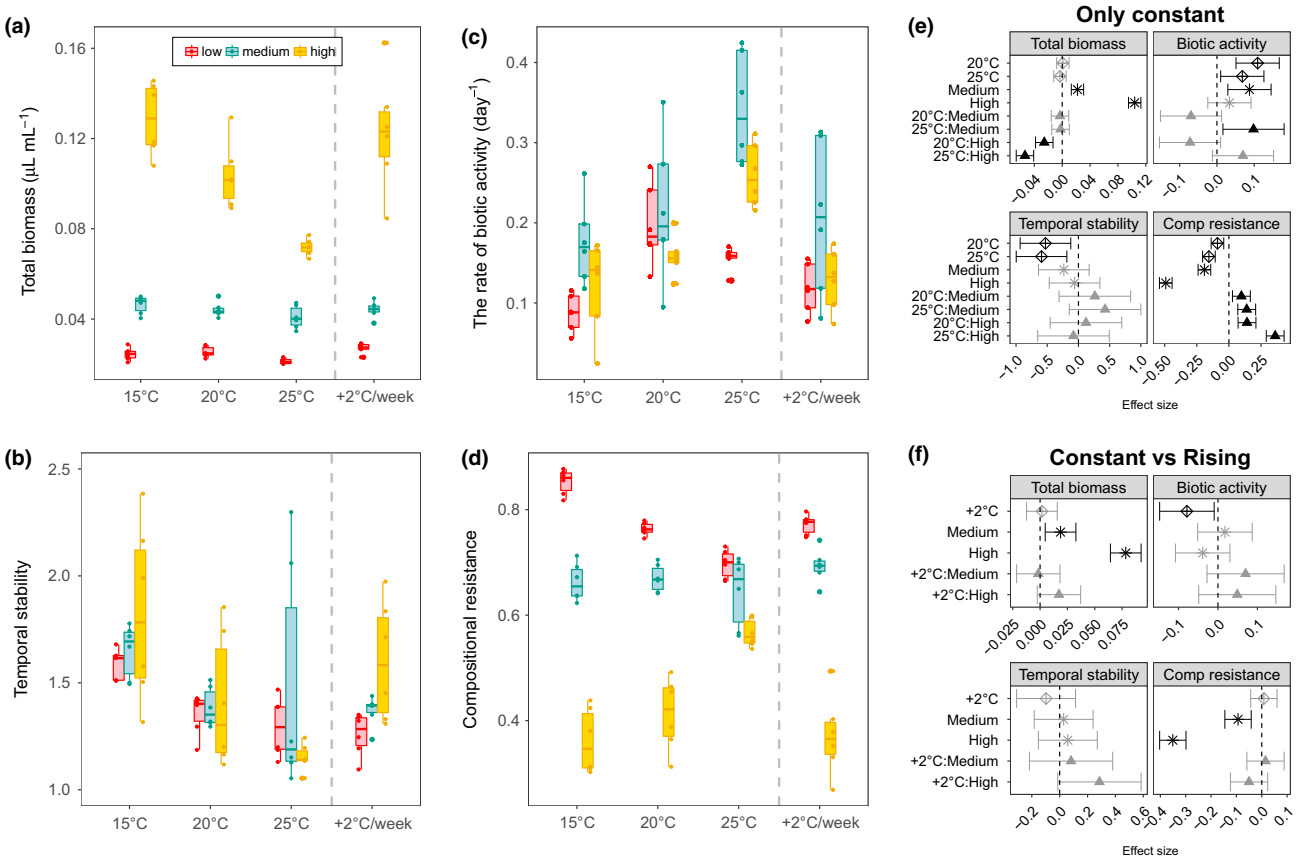


**Figure 2** Community and ecosystem properties of the experimental food web. (a) depicts the averaged total biomass through time, (b) shows temporal stability of the total biomass, (c) is the mass-corrected rate of biotic activity which shows the speed at which the mass-corrected dissolved oxygen (µmol µL$^{-1}$) level increases per day and (d) is the compositional resistance for each community by treatment. Panel (e) and (f) show the effect sizes calculated from linear model comparison. Panel (e) shows the effect sizes across constant temperature levels and nutrient levels (being the combination of constant 15 °C and low nutrient levels the baseline). Panel (F) depicts effect sizes for the rising + 2 °C per week and constant 20 °C combined with all nutrient levels (being the combination of 20 °C and low-nutrient level the baseline). Error bars correspond to 95% confidence intervals.

Enrichment tended to increase body size, though with some exceptions: *Colpidium* at 25 °C exhibited a hump-shaped effect of enrichment, and show rather larger body size in higher temperature and also in higher enrichment. We found a large negative interactive term in high enrichment and 25 °C. *Dexiostoma*, on the other hand, decreased in body size across temperature in constant treatments and enrichment affected body size positively. Interactive effects were not strong. Body size was larger in the rising temperature treatment than in the 20 °C treatment for *Dexiostoma* (Fig. 4c, Table S8).

### Model robustness and putative empirical mechanisms

The sensitivity analysis showed that the basal species is insensitive to the activation energy of carrying capacity, but shows negative correlation (−0.49) with its scaling coefficient. In general, changes in the activation energy parameters in the model have only small impacts on any of the state variables (Table S11). Furthermore, the model assumes no temperature dependence of biomass assimilation efficiency. Results show that all species are insensitive to both parameters of assimilation efficiency. The model assumes that the carrying capacity of the basal species increases linearly with nutrient enrichment and decreases exponentially with warming. Our results show that bacteria biomass indeed increases with nutrient enrichment, but we could not detect a consistent response to temperature (Fig. S6).

## DISCUSSION

### The interactive effects of temperature and nutrient enrichment

While additive effects largely prevailed, we observed antagonistic interactions between high temperature and high enrichment for the majority of response variables (Table 2). In contrast, at 15 and 20 °C with low and medium nutrient enrichment additive effects dominated. Interactive effects increased the further the communities were moved away from their reference environmental conditions. Species can be buffered against a certain degree of environmental change, by means of behavioural or physiological changes, but these mechanisms may break down with a sufficient degree of environmental change. The occurrence of interactive effects also showed variation across the different levels of ecological organisation. Whereas the population level responses are non-additive, especially in the high temperature and high

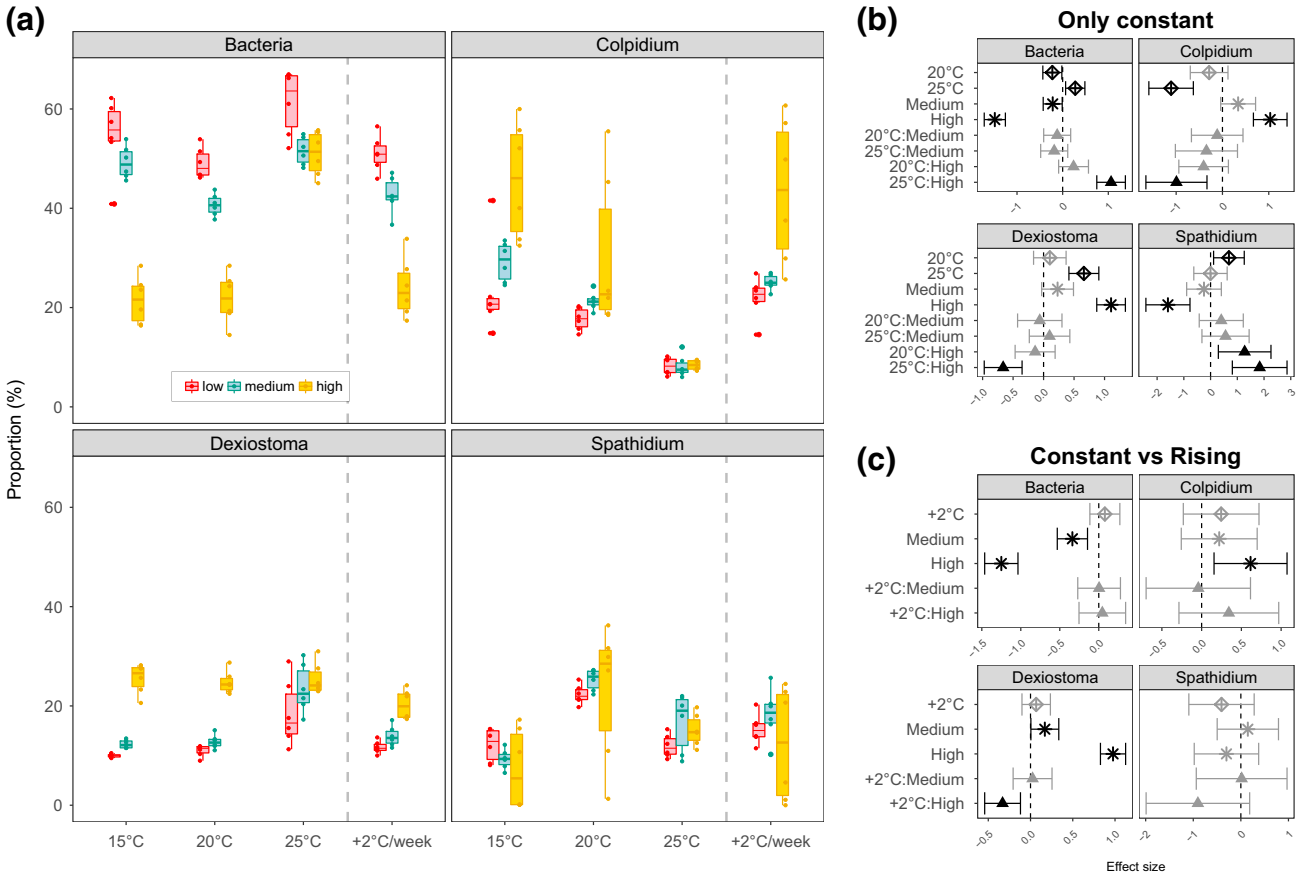


**Figure 3** Proportion of biomass of major groups by treatment. (a) shows how the average proportionality of each trophic level changed in the experiment. Panels (b) and (c) show the effect sizes extracted from beta regression models comparing (b) only constant temperature levels and nutrient levels (being the combination of constant 15 °C and low nutrient levels the baseline) and (c) the rising + 2 °C per week and constant 20 °C combined with all nutrient levels (being the combination of 20 °C and low nutrient level the baseline). Error bars correspond to 95% confidence intervals.

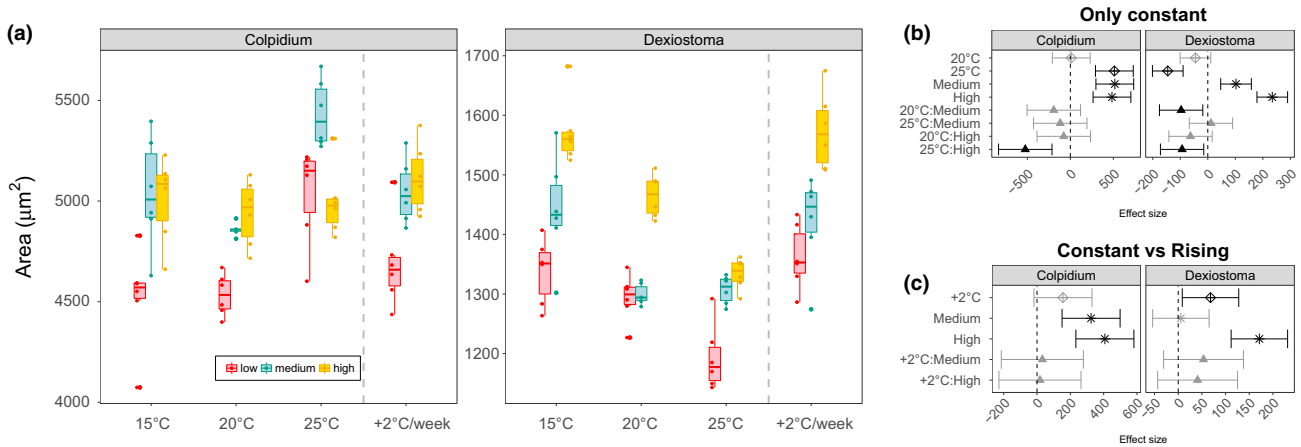


**Figure 4** Body size distribution of consumer species. (a) shows the averaged body size over the first 10 days of the experiment for both consumer species. (b) and (c) show the size effects extracted from linear regression models, where (b) shows constant temperature levels and nutrient levels (being the combination of constant 15 °C and low nutrient levels the baseline) and (c) compares the rising + 2 °C per week and constant 20 °C combined with all nutrient levels (being the combination of 20 °C and low nutrient level the baseline). Error bars correspond to 95% confidence intervals.

enrichment combination, properties on lower (e.g. individual body size) and higher levels of organisation (e.g. ecosystem level) respond in an additive fashion. Population biomass of all species was non-additive in the high enrichment treatments regardless of the temperature. These responses on the population-level are reflected in highly non-additive response of the

compositional resistance (i.e. negative synergistic interaction) and the non-additive (i.e. negative antagonistic) response of total biomass to high enrichment. Biomass was reduced in high-temperature treatments (20 and 25 °C) with high enrichment. The temporal stability of biomass, however, responded additively to temperature and enrichment in all treatment combinations. Individual body sizes of both consumer species show mostly additive effects. At the ecosystem level, there was no antagonistic interactive effect of high temperature and high warming; however, temperature and enrichment increased the rate of biotic activity additively, in line with expectation based on metabolic theory (O'Connor *et al*. 2009).

If interactive effects of temperature and enrichment are caused predominantly by mechanisms operating at the individual level, that is responses of individuals, and such effects decrease in influence moving from individual to population to community to ecosystem level, one might expect these results, though the generality of this pattern deserves further attention.

### Comparison to model predictions and empirical findings

The allometric and temperature-dependent modelling work (Binzer *et al*. 2012) provides quantitative predictions across levels of organisation that can be compared with our findings and previous empirical results.

(1) Predictions that temperature decreases and nutrient enrichment increases carrying capacity is partially supported as bacterial biomass was affected by enrichment, but not temperature. Previous studies found a variety of responses: mesocosms mimicking shallow lakes also reported positive nutrient but no temperature effects on total algal and macrophyte biomass (McKee *et al*. 2003; Moss *et al*. 2003). Özen *et al*. (2013) found no effect of temperature, but both positive effects of nutrients, and their interaction on microbial biomass, whereas positive effects of nutrients and warming were reported by Moghadam & Zimmer (2016). Other studies found mostly differences due to temperature: Ventura *et al*. (2008) reported lower primary producer biomass with warming but not enrichment. Sea weed biomass was negatively impacted by warming but when applied jointly with enrichment led to even higher decrease (Werner *et al*. 2016). O'Connor *et al*. (2009) on the other hand described pronounced changes in phytoplankton and bacterial biomasses with warming and nutrient addition; bacterial biomass increased with warming, while phytoplankton biomass decreased due to increased pressure by grazers. Finally, a study of pond food webs found that warming produced top-heavy and enrichment induced bottom-heavy food webs and that enrichment increased biomass across all trophic levels, whereas warming reduced the biomass of autotrophs without affecting consumers (Shurin *et al*. 2012). The diversity of responses highlights the need for community models in predicting the response of a specific species/level in a complex community.

(2) We expected an interacting effect between high enrichment and high temperature on total community biomass, which was supported by the data. Enrichment reduced the effect of warming, but was not strong enough to cancel the effect of warming, resulting in a negative antagonistic effect. This result mirrors findings by O'Connor *et al*. (2009) where the total biomass of the food web was increased by nutrient addition, but declined with warming despite increases in primary productivity. As in our case, warming increased consumer biomass relative to resource biomass.

(3) The predicted decrease in stability at high enrichment and low temperature was partially met. Temporal stability of community biomass was highest at the lowest temperature, but not different among enrichment levels, contrasting findings by Kratina *et al*. (2012) reporting lower stability of chlorophyll a concentration in a mesocosm experiment with nutrient

**Table 2** Summary of the estimated interaction types. All estimated individual and interaction effect sizes for all response variables (T: temperature, N: nutrient enrichment, TxN: temperature and nutrient interaction) and their classification *sensu* Piggott et al. (2015); additive (AD), positive antagonistic (+A), negative antagonistic (−A), positive synergistic (+S).

| | 20°C:medium | | | | 25°C:medium | | | | 20°C:high | | | | 25°C:high | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Response | T | N | TxN | Class. | T | N | TxN | Class. | T | N | TxN | Class. | T | N | TxN | Class. |
| Body size | | | | | | | | | | | | | | | | |
| Colpidium | 0 | + | 0 | AD | + | + | 0 | AD | 0 | + | 0 | AD | + | + | – | +A |
| Dexiostoma | 0 | + | – | +A | – | + | 0 | AD | 0 | + | 0 | AD | – | + | – | +A |
| Biomass ratio | | | | | | | | | | | | | | | | |
| Bacteria | – | – | 0 | AD | + | – | 0 | AD | – | – | 0 | AD | + | – | + | −A |
| Colpidium | 0 | 0 | 0 | AD | – | 0 | 0 | AD | 0 | + | 0 | AD | – | + | – | +A |
| Dexiostoma | 0 | 0 | 0 | AD | + | 0 | 0 | AD | 0 | + | 0 | AD | + | + | – | +A |
| Spathidium | + | 0 | 0 | AD | 0 | 0 | 0 | AD | + | – | + | −A | 0 | – | + | −A |
| Population biomass | | | | | | | | | | | | | | | | |
| Bacteria | 0 | + | 0 | AD | 0 | + | 0 | AD | 0 | + | – | +A | 0 | + | + | +S |
| Colpidium | 0 | + | 0 | AD | 0 | + | 0 | AD | 0 | + | – | +A | 0 | + | – | +A |
| Dexiostoma | 0 | + | 0 | AD | 0 | + | + | +S | 0 | + | 0 | AD | 0 | + | – | +A |
| Spathidium | 0 | 0 | 0 | AD | 0 | 0 | 0 | AD | 0 | 0 | + | +S | 0 | 0 | 0 | AD |
| Total biomass | 0 | + | 0 | AD | 0 | + | 0 | AD | 0 | + | – | +A | 0 | + | – | +A |
| The rate of biotic activity | + | + | 0 | AD | + | + | + | +S | + | 0 | 0 | AD | + | 0 | 0 | AD |
| Temporal stability | – | 0 | 0 | AD | – | 0 | 0 | AD | – | 0 | 0 | AD | – | 0 | 0 | AD |
| Compositional resistance | – | – | + | −A | – | – | + | −A | – | – | + | −A | – | – | + | −A |

addition. Two potential explanations can be invoked to explain this difference: (3.1) We did not observe oscillations or extinctions of the intermediate level. Instead, at high-nutrient level, the basal resource had the lowest and consumers had the highest share in total biomass which points to biomass accumulation at intermediate level in the food chain. In contrast, Kratina *et al*. (2012) found that enrichment led to higher, asynchronous fluctuations, partly caused by algal blooms. Fluctuations were counteracted by simultaneously warming the mesocosms which triggered stronger top–down effects. Their findings are hence in line with the paradox of enrichment (Rosenzweig 1971). (3.2) On the other hand, Binzer *et al*. (2012) assume that nutrient-rich environments save species from warming-induced starvation, that is at high-temperatures consumers in nutrient-poor communities run a risk of starvation because of an unfavourable ratio of ingestion and metabolic rate. We indeed observed a temperature-induced structural shift on the resource level between high and low enrichment at high temperature. This implies that the increase in resource production was larger than the metabolic rate of consumers; therefore the biomass was able to accumulate at the bottom of the food chain.

(4) The prediction that individual cell size decreases with temperature, exacerbated by low-resource availability, was met by *Dexiostoma*, but not *Colpidium*. *Colpidium* increased in body size with enrichment and temperature. The thermal range of *Colpidium* provides a potential explanation: temperatures above 21 °C fall into the (sub)-lethal range for *Colpidium*, where different responses are possible (Atkinson *et al*. 2003). Previous studies on changes in size in food webs reported no effect of warming nor enrichment on zooplankton mean size (Kratina *et al*. 2012) or on caddisfly body size (Hines *et al*. 2016).

### The right answer for the right reasons?

Some of the assumptions of the model such as the lack of temperature-independence of biomass assimilation efficiency (Lang *et al*. 2017; García-Carreras *et al*. 2018), the temperature dependence of carrying capacity (DeLong 2014; Bernhardt *et al*. 2018), the temperature dependence of attack rate (Dell *et al*. 2014), or a single fixed activation energy across species (Dell *et al*. 2011) can be challenged. The close qualitative match of predictions and observations suggests limited importance of deviations from these assumptions and/or good match between these assumptions and features of the experimental system, such as a structured body size distribution. Our sensitivity analysis shows very little effect of the temperature dependence of the carrying capacity on state variables. Similarly, the activation energy of all parameters has little to no impact on state variables in the model; therefore differences between species can probably be ignored as well. We could not test for the temperature dependence of the attack rate, but we acknowledge that might have an effect of the model outcome even though some previous empirical research did not find a relationship between temperature and attack rate (Rall *et al*. 2012).

While the model incorporates a food chain, our system deals with additional competition on the consumer level. Other assumptions of the model such as Type II functional responses might differ from the functional responses in the experimental system. Furthermore, assumptions such as hump-shaped relationship between mass- and temperature-dependence of attack rate and handling time may not be met by the experimental system. There is also a time scale difference between model and experiment: model predictions are at equilibrium around 10 000 years, while the experiment only lasted for hundreds to dozens of generations, depending on the trophic level. This would raise the possibility that experimental results are transient. The analysis of the effects of enrichment and warming through time suggests that there is a short transient phase over the first days of the experiment; however, effects are consistent thereafter.

In total, there are many possible differences between the model assumptions and the biology of the experimental system and some of these differences may be even unknown. Yet, the predictions of the model match the empirical results. While we cannot rule out that some effects are due to alternative mechanisms, we suggest that the match is due to the robustness and generality of the predicted and observed effects of temperature and nutrient enrichment on ecological community structure and dynamics.

### Constant vs. rising temperature

Although climate change is affecting the environment and embedded ecosystems through gradual warming, ecologists most commonly test the effect of warming by treatments of constant elevated temperature. We explicitly tested whether communities at constant elevated temperatures show similar responses than gradually warmed communities. Overall, we found that the gradually warmed community did show similar responses in terms of community response variables averaged through time. Only on the individual level, we detected a larger average size in the rising compared to the constant treatment. Our results hence expand the findings of Fox & Morin (2001). These results suggest that the common practice of using constant temperature to mimic the effects of temperature change on communities and populations is valid, at least in the sub-lethal temperature range used in our experiment, and if gradual change is slower than the generation time of the focal organisms. How communities can respond to lethal temperatures is currently an open question which can be addressed in the wider context of community evolutionary rescue (Gonzalez *et al*. 2013).

## CONCLUSION

Our study revealed when warming and enrichment interact across levels of organisation. Although interactions occurred frequently and are expected to render predictions more difficult, our observations were generally well captured by a theoretical model that integrates interdependencies among trophic levels. Considering responses from the individual to the ecosystem level helped us to understand how changes at one level affect higher or lower ecological levels. Whereas challenging, considering these pathways in natural ecological systems is critical to understand and predict the implications of ongoing environmental change.

## ACKNOWLEDGEMENTS

AT, OP and FP were financially supported by Swiss National Science Foundation Grant 31003A_159498.

## AUTHORSHIP

AT, OP, FP conceived the study; AT collected and analysed the data; and AT, OP and FP interpreted the data. AT and FP wrote the first draft of the paper. All the authors contributed to revisions of the paper.

## DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.5470b62.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Editor, Tadashi Fukami
Manuscript received 28 August 2018
First decision made 28 September 2018
Second decision made 3 January 2019
Manuscript accepted 1 March 2019