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Temperature and trophic structure are driving microbial productivity along a biogeographical gradient

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Temperature is known to influence ecosystem processes through its direct effect on biological rates such as respiration and nutrient cycling. These changes can then indirectly affect ecologically processes by altering trophic dynamics, the persistence of a species in a given environment, and, consequently, its distribution. However, it is not known if this direct effect of temperature on biological rates is singularly the most important factor for the functioning of ecosystems, or if trophic structure and the adaptation of a species to the local environment also play an essential role. Understanding the relative importance of these factors is crucial for predicting the impact that climate change will have on species and ecosystems. To achieve a more complete understanding of the impact of changing temperatures, it is necessary to integrate perspectives from biogeography, such as the influences of species distribution and local adaptation, with ecosystem and community ecology. By using the microbial community inhabiting the water-filled leaves of *Sarracenia purpurea*, we tested the importance of temperature, trophic structure, and local adaptation on ecosystem functioning. We accomplished this by collecting communities along a natural temperature gradient and maintaining these communities in a common garden, factorial experiment. To test for the importance of local adaptation and temperature, the origin of each community was crossed with the temperature from each site. Additionally, to test the importance of top-down trophic regulation for ecosystem functioning, the presence of the mosquito larvae top predator was manipulated. We found that temperature has a greater effect on ecosystem functioning than origin, and that top-down trophic regulation increased with temperature. Our results emphasize the synergistic effects of temperature and biotic interactions when predicting the consequences of global warming on ecosystem functioning.

Forecasting the impact that climate change will have on the functioning of ecosystems is a notoriously difficult scientific challenge due to the multiple dimensions of the problem. Temperature is known to directly influence the physiology of organisms by changing their metabolic rates, and thus their production (Brown et al. 2004). These effects can then propagate to the population- and community-level, influencing species interactions and distributions, and ultimately, community composition and function. To date, these perspectives have been studied independently (Massol et al. 2011), on one hand, by ecosystem and community ecologists (Jochum et al. 2012, Yvon-Durocher et al. 2012), and on the other, by biogeographers (Pereira et al. 2010). As climate change is predicted to accelerate the rate at which novel ecosystems will emerge (Lurgi et al. 2012), it is now important to integrate these perspectives into a coherent framework and to disentangle their effects on ecosystem functioning.

Ecologists examining the effect of climate change on ecosystem processes have empirically (Huston and Wolverton 2009, Pomati et al. 2012) and experimentally (Blankinship et al. 2011, Ott et al. 2012, Wernberg et al. 2012) demonstrated that respiration, biomass accumulation, and nutrient cycling vary along temperature gradients. These results have been pivotal for providing evidence that rates of ecosystem processes are likely to increase with temperature (Yvon-Durocher et al. 2010). However, most of these studies have not taken into account the distribution of taxonomic and functional diversity along temperature gradients. At most,

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common garden experiments have tested for local adaptation to temperature at the species-level (Savolainen et al. 2007), but have not considered the effects on species interactions, and thus the possible effects of changing ecological dynamics on ecosystem functioning. Given the key role that top predators have on several ecosystem functions (Zarnetske et al. 2012) and their well-documented vulnerability to environmental changes (Estes et al. 2011, Worm and Tittensor 2011), it is essential to understand how temperature-dependent trophic control will be altered by climate change. Theory predicts that ecological interactions are temperature-dependent (Dell et al. 2014), but it is unclear how temperature will change trophic regulation (i.e. top-down and bottom-up control, Shurin et al. 2012), and ultimately, how this will affect ecosystem processes.

Our understanding of how climate change will affect ecosystem functioning is also made more difficult because ecological theories have remained unlinked. For example, the metabolic theory of ecology (Brown et al. 2004) states that temperature is a key driver of ecosystem functioning and may supersede the effects of community composition and structure. It predicts that, all else being equal, warmer environments should have higher turnover and productivity to satisfy increasing metabolic demand, regardless of local species composition. Yet, according to the temperature-dependent consumer-resource theory (Vasseur and McCann 2005), consumption and respiration rates will scale exponentially with increasing temperature, thus increasing the top-down regulation of predators when temperature increases. Community composition may therefore also significantly affect ecosystem functioning over temperature gradients.

In contrast, approaches relying on the species' niche and competitive interactions suggest that an individual should perform best at the temperature found in its site of origin, due to local adaptation at the species-level (Kawecki and Ebert 2004) and species-sorting at the community-level (Leibold et al. 2004), and it should significantly underperform as it moves away from this optimal temperature. Consequently, it can be predicted that ecosystem functioning will decline when a locally-adapted species is no longer in its optimal thermal conditions (Mouquet and Loreau 2003). This phenomenon can be demonstrated by the biogeographical species response under climate change. With climate change, the temperature at the site of origin can increase, thus causing locally-adapted species to either shift their ranges to new habitats (Parmesan 2006, Rosenzweig et al. 2008) or the extinction of species who are unable to migrate. The structure of entire food webs will thus be altered by the direct and indirect effects of these species-level changes (Gilman et al. 2010, Albouy et al. 2014), potentially affecting the spatial distribution of ecosystem processes (Poisot et al. 2013).

In addition to the diverse predictions from these theories, theoretical studies usually consider a maximum of two trophic levels to address the impact of temperature on ecosystem functioning (Vasseur and McCann 2005). It is therefore unclear if the mechanisms involved in each of these theories are distinct, or if they interact, creating a synergistic effect on ecosystem functions in large food webs. There is therefore a need to integrate ecosystem and community ecology with species distributions, and thus biogeography, (Cheung et al. 2013) into an operational framework to predict how changes in temperature affect ecosystem-level processes (Fig. 1A).

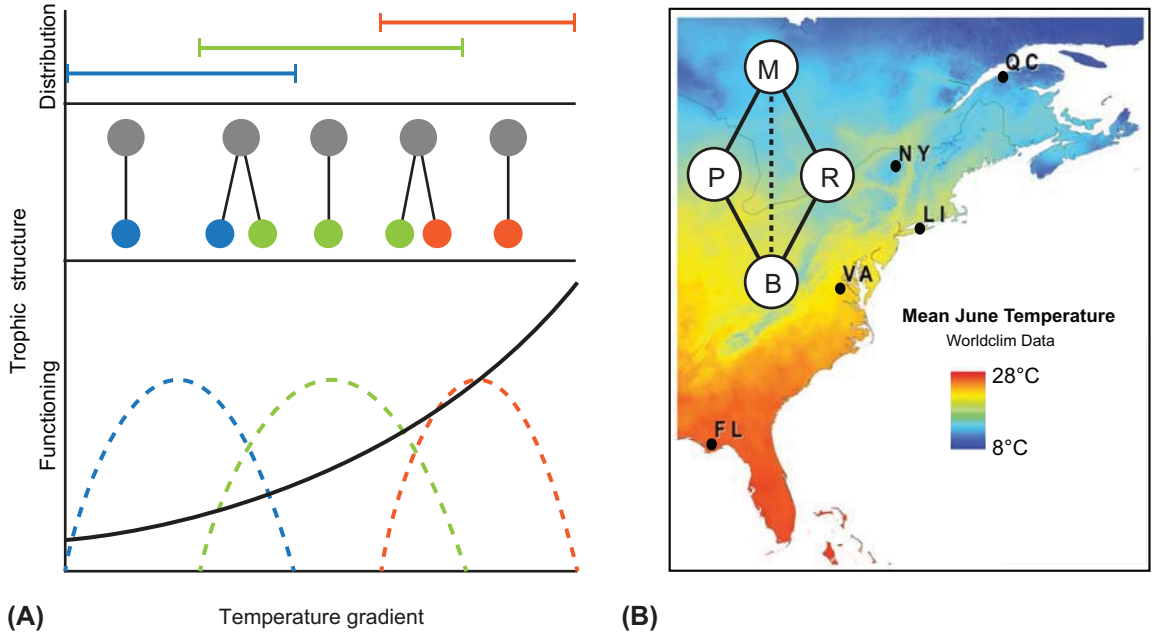

Figure 1. Conceptual context of the study. (A) Along a temperature gradient (blue being the coldest and orange the warmest), species with different niche requirements will occupy different positions (as indicated by the horizontal lines, top panel). The structure of the food web may display some turnover (depicted in the middle panel) along the gradient, which will result in a varying trophic control with temperature. In addition, the expected ecosystem functioning along the gradient will depend on the relative importance of the direct effect of temperature on metabolic rate (solid black line, bottom panel). (B) Specific context of the study. The map shows the average daily temperature in June over North America according to WorldClim, with the 5 sampling sites indicated by black dots. The overall food-web structure is inserted in the map: mosquito larvae (M) feed on protists (P) and rotifers (R), which consume the bacteria (B).

Our objective in this study was to evaluate the relative importance of temperature, local adaptation and community structure on the functioning of a microbial ecosystem. We accomplished this with an experiment using the natural aquatic ecosystem held within the leaves of the carnivorous pitcher plant *Sarracenia purpurea*, taking advantage of its wide distribution along a natural temperature gradient from the southeastern United States to northern Canada (Fig. 1B, Buckley et al. 2003). This system is ideal for this analysis, as it allows testing of the interaction between different ecosystem-level effects of temperature on community composition. Moreover, this system makes it easy to conduct controlled common-garden transplant experiments on entire ecosystems (Strivastava et al. 2004), which are important for identifying the response of communities to displacement along a gradient and to compare this response to theoretical expectations under the different scenarios.

Our experiment consisted of a common-garden, factorial design in which we manipulated temperature, origin, and trophic structure to determine their importance for the ecosystem processes of bacterial respiration and density, and the production of nitrogen and phosphorus. In the Supplementary material Appendix 1, we explored the consequences of different assumptions on the shape of the temperature response curve with a simple, parameterized, consumer-resource model representing the *S. purpurea* food web. We predicted that if temperature is the most important factor affecting ecosystem functioning, our results would show that, irrespective of the environment in which community assembly took place, warming would result in increased ecosystem functioning (Fig. 2A). Yet if temperature-dependent trophic control was an important factor altering ecosystem functioning, our experimental results would demonstrate that the consequences of removing a top predator on ecosystem processes should increase with temperature. Alternatively, if local adaptation is more important for ecosystem functioning than temperature or temperature-dependent trophic control, functioning would be highest in the environment that the community has originated from (Fig. 2B). This would mean that, for any given community, moving away from its optimum temperature (the temperature the species originated from) would result in a maladaptation. Consequently, we predicted that a community that is assembled under a given temperature will have its functioning decrease as temperatures either increase or decrease.

Methods and material

Study system

Sarracenia purpurea is a carnivorous plant found in nutrient-poor habitats throughout much of the eastern United States and Canada (Buckley et al. 2003). The open leaves of this plant form a pitcher-shape and fill with rainwater. This leaf structure is a passive trap that attracts insects, which ultimately drown in the water-filled habitat. An aquatic inquiline food web develops in each leaf and uses the insects as basal resources. This food web is composed of a core group of obligate species that have evolved specific requirements for survival in the host plant and a larger set of generalist taxa who are commonly found within the *S. purpurea* ecosystem throughout the plant's geographic range, but whose occurrence is influenced by both stochastic and deterministic

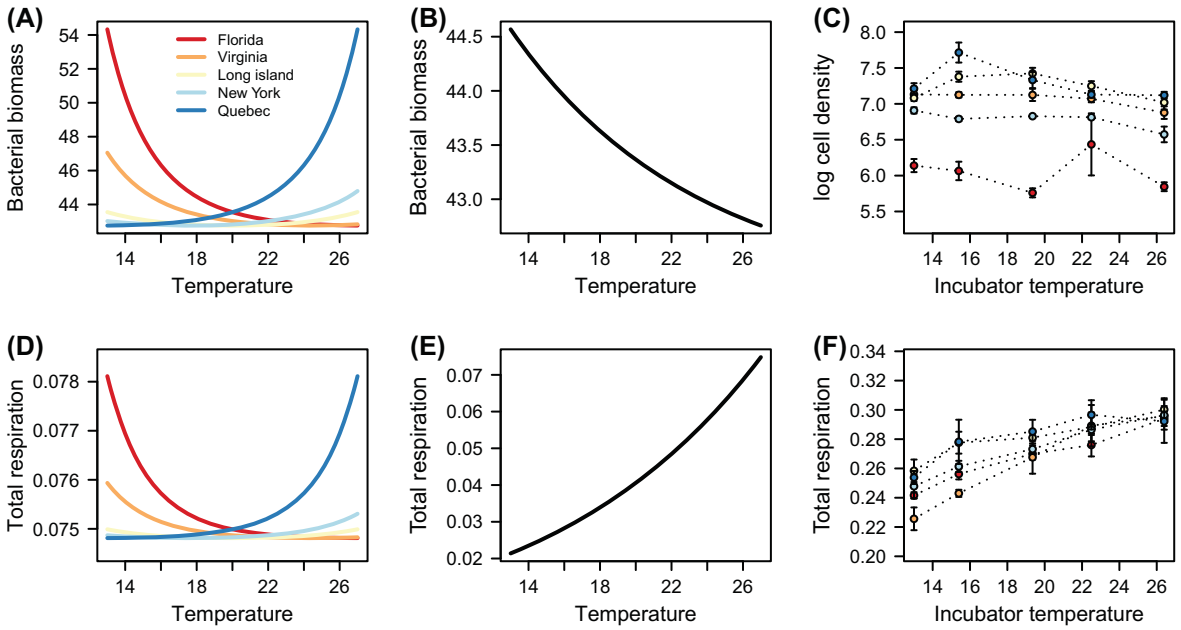

Figure 2. Comparison of the theoretical (Supplementary material Appendix 1) and empirical results for bacteria density (A, C) and total respiration (D, F). Results are presented in the presence of mosquito larvae (i.e. trophic control). (A, D) are the predictions assuming bacterial species-sorting in which the mean temperature for the warmest to coldest sites, respectively, are 26.4, 22.5, 19.35, 15.4, and 13°C and the model is solved with all trophic levels included. (B, E) are the predictions assuming metabolic scaling. (C, F) are the experimental results (see Table 1 for the ANCOVA results). Units are in µg C/l for model predictions of bacteria biomass, log cell density/ml for empirical bacterial density, µg C/day for model predictions of respiration, Δ absorbance (nm) for empirical respiration, and °C for average daily temperature. The 'no top predator' data are provided in the Supplementary material Appendix 1.

dynamics (Buckley et al. 2003, Baiser et al. 2012). The main food web consists of bacteria and yeast as the bottom trophic level, protozoans and rotifers (*Habrotrocha rosa*) as the intermediate trophic level, and the larval stage of the endemic mosquito *Wyeomyia smithii* as the top predator. A midge species (*Metriocnemus knabi*) can also be found within the food web, helping to facilitate the decomposition of the insects (Heard 1994). Other species, such as mites, copepods, and larvae of the flesh fly *Fletcherimyia fletcheri*, can be present but are much less common than the other members in the food web. At low taxonomic resolution (e.g. bacterial morphotype, protozoan genera), the food web structure is highly conserved across the geographic range of the plant (Buckley et al. 2003, 2010, Baiser et al. 2012). In addition, the plant may have evolved a mutualistic relationship with some elements of the food web, allowing for efficient nutrient cycling within the plant, particularly in terms of mineralizing the insect prey and producing nitrogen for the plant (Mouquet et al. 2008). While the plant is nitrogen-limited and relies on the inquiline community to provide the much needed nitrogen, the community is thought to be mainly carbon- and phosphorus-limited and obtains these nutrients by decomposing the insect prey (Gray et al. 2006). This system has been used for decades as a model system to address multiple ecological questions both experimentally (Addicott 1974, Kneitel and Miller 2002, Hoekman 2010, terHorst 2010) and theoretically (Mouquet et al. 2008).

Experiment

We experimentally tested the importance of temperature, trophic structure, and local adaptation on ecosystem functioning. We accomplished this by collecting communities along a natural temperature gradient and maintaining these communities in a common garden, factorial experiment, crossing origin with the temperature from each site. Additionally, to test the importance of top-down trophic regulation for ecosystem functioning, we adjusted trophic structure in all treatments by having a top predator either present or absent in each community.

Sampling locations

Entire aquatic inquiline communities of *S. purpurea* were collected simultaneously from five sites (Florida to Québec) across the latitudinal gradient of the plant's native distribution. Sites were chosen to provide a range in average maximum and minimum June temperatures from the southern-most selected site to the northern-most selected site (data acquired from WorldClim; <www.worldclim.org>), permit availability to sample, and precipitation (needed for forming *Sarracenia*'s inquiline communities). WorldClim data provide an indication of the regional average temperature regime, and differences in temperature among sampling locations are much greater than the expected micro-climatic variation within and among bogs from a region. The following sites and maximum/mean/minimum June temperatures were used for this experiment: Sumatra, Florida (32.1/26.4/20.6°C) as the southern-most site; Ruther Glen, Virginia (28.8/22.5/16.2°C); Riverhead, New York (24.4/19.35/14.3°C); Newcomb, New York (22.2/15.4/8.6°C); and Saint-Noël, Québec (19/13/7°C) as the northern-most site. A map containing the sites and temperature gradient is in Fig. 1B.

Inquiline community field collection

Sampling was conducted simultaneously on 5 July 2011 across five sites using a standardized protocol. Sterile pipettes were used to sample communities from randomly selected new leaves that had opened within the past month. At each field site, the communities in each randomly selected leaf (~ 10–20 ml) were gently mixed and pooled together into an autoclaved bottle until 3 l of water were collected per site (approximately 200–300 leaves per site). Precautions were taken to avoid leaves containing cloudy or pink water which is indicative of unusually large prey capture, resulting in extremely high bacterial input. Such communities are anaerobic and thus could have confounded the results of the experiment. Using a sterilized pipette, mosquito larvae were carefully separated from the aquatic community and placed into a separate autoclaved nalgene bottle containing 800 ml of pitcher plant water. Both bottles (mosquito larvae absent and mosquito larvae present) were chilled on ice packs and sent to the Univ. du Québec à Rimouski where the experiment took place.

Experimental set-up

All samples from all sites remained cooled for the same amount of time (48 h) before initiating the experiments in order to slow biotic interactions and to avoid overheating during transportation. This procedure allowed all treatments to experience the same initial conditions and is a common practice used in *Sarracenia* research (Paisie et al. 2014, Zander et al. 2015). Water from all five sites was filtered with a large, sterilized mesh size (2 mm) to remove the midge (*Metriocnemus knabi*) and flesh fly larvae (*Fletcherimyia fletcheri*), and detritus, in order to create homogenized nutrient availability in each of the five communities. What remained in the community were bacteria in the bottom trophic level and protists and rotifers in the intermediate trophic level. Mosquito larvae were subsequently re-introduced to half of the communities as a treatment.

To homogenize initial bacterial density for each of the five communities, aliquots of samples were used to perform flow cell cytometry (according to Belzile et al. 2008). Bacterial density was then standardized across sites by adjusting the volume of the communities with autoclaved deionized water, resulting in 1.09×10^7 bacteria per ml for each origin. This technique is standard for research conducted on the *Sarracenia* system, as it has been shown that the members of the food web are able to easily survive and maintain normal interactions, even when placed directly into deionized water (Kneitel and Miller 2003, Hoekman 2011, Gray 2012, Kadowaki et al. 2012, Kneitel 2012, Zander et al. 2015). As a control, an equal volume of water from each of the five sites was also pooled to make the 'mixed origin' community.

Using a sterile glass pipette, we placed 20 ml aliquots of water from each of the five original field sites and the mixed community into 50 ml sterilized macrocentrifuge tubes. These tubes were used because their shape best mimics the shape and volume of *Sarracenia purpurea* leaves. A solution containing a constant 6 mg of autoclaved Tetramin fish food

(Tetra Holding, Blacksburg, VA) was added to each tube in order to completely standardize the amount of resource input for the bacteria across each replicate and each treatment. This preparation is a reasonable approximation of the energy source that occurs naturally when insects fall within the plant's pitcher-shaped leaf, has been shown to produce similar quantitative results (terHorst 2010, terHorst et al. 2010), and allowed for a more precise addition of resources than if individual insects had been used. Autoclaved glass beads (2 ml, each 3 mm diameter with 1 mm hole in the center) were added to mimic habitat heterogeneity produced by the exoskeletons of insects and leaf detritus that are found at the bottom of *S. purpurea* leaves (terHorst 2010). Additionally, opaque paper was wrapped around each tube (from the 5–25 ml mark) to replicate the light availability inside a *S. purpurea* leaf.

The experiment was a fully-factorial common-garden design crossing five temperature treatments, the six communities (five origins and the mixed origin community) and the presence/absence of the predatory mosquito larvae (Supplementary material Appendix 1, Table A1). The communities assigned to the 'top predator' treatment contained two 3rd instar larvae of the pitcher plant mosquito *Wyeomyia smithii*. The larvae were double rinsed in sterile deionized water before they were placed into their designated test tubes. Except for the 'mixed origin' treatment, the selected larvae were from the same origin as the community in which they were placed. For the 'mixed origin' treatment, mosquito larvae that were collected from the mid-range of the geographic distribution (Long Island, NY) were used. Each treatment was replicated five times for a total of 300 communities (Supplementary material Appendix 1, Table A1).

Five incubators (Sanyo MIR-154) were used to reproduce the mean June temperatures at each site (one incubator per site) for the temperature treatment. For each incubator, the mean maximum and minimum June temperatures from one of the five field sites were used to run a 12 step program with a daily incremental increase in temperature from 6:00 to 16:00 h and an incremental decrease in temperature for the remainder of the 24 h period. A 12 h light:dark cycle was maintained throughout the course of the experiment to standardize the amount of light input in each incubator. Mosquito larvae were checked daily during the course of the experiment and those that either pupated or died were replaced with another mosquito larva from the same origin. The selected replacement mosquito larvae had been maintained in incubators matching temperatures from their origins.

The experiment was conducted over 6 d, which is equivalent to approximately 18 to 24 generations of protozoans (Lüftenegger et al. 1985) and 48 generations of bacteria (Gray et al. 2006). The experiment ended when the bacteria and protozoans were expected to reach their carrying capacities in this system (Gray 2012, Kadowaki et al. 2012). The fast generation time of microbial systems thus allows experiments to be conducted over a short amount of time, while producing results equivalent to longer-term experiments on larger organisms (Srivastava et al. 2004). The 3rd instar mosquito larvae also pupate after approximately one week (Bradshaw and Holzapfel 1990). Therefore, if the experiment was conducted for longer than 6 d, the top predator would have had to be replaced at least once in all replicates, adding unnecessary variation. In addition, if resources were added on a weekly basis, this closed system could become anaerobic, thus skewing our results. The duration of the experiment was also set to avoid that the food web would evolve to laboratory conditions and thus buffer the effect of the origin (terHorst 2010).

Ecosystem functioning

Ecosystem functioning was quantified for all communities at the end of the experiment as cell respiration, phosphorus and ammonium production, and bacterial density. We used a MicroResp system (The James Hutton Inst., Scotland) to measure bacterial respiration, using a method adapted from the manufacturer's protocol. In brief, a 1 ml aliquot of the inquiline community was extracted from each tube and placed into 96 deep-well plates (1 plate per samples within an incubator). The plates were then covered with a seal provided by the manufacturer and a microplate consisting of an indicator dye composed of agar, cresol red and a potassium chloride- based reactive solution was placed over the seal so that each sample was able to respire into the dyed agar (Campbell et al. 2003). This indicator dye changes color with the reaction of CO_2 with bicarbonate. Measurements of the indicator dye color were taken at T0 (before the samples were allowed to respire onto their respective agar-filled well). They were then placed into their respective incubators. Measurements were taken again after the communities respired for 48 h. Respiration was quantified by taking the difference in light intensity at 570 nm at time 0 and after 48 h of respiration. Light intensity was measured with an absorbance spectrophotometer microplate reader (Biotek Instruments, USA).

An additional 2 ml of the inquiline community water was extracted, filtered on a heated GF/F 0.7 µm filter (heated at 500°C for 2 h), and frozen at –20°C until processing. Phosphorus was determined using a standard phenol and sodium citrate oxidation method (Parsons et al. 1984). Ammonium concentration was determined using a fluorometric method (Holmes et al. 1999) in which a single working reagent (composed of orthophtaldialdehyde, sodium sulfite, and sodium borate) is combined with each sample and measured with a spectrofluorometer. An additional 0.1 ml aliquot of each sample was fixed with glutaraldehyde (0.1% final concentration) and stored at − 80°C before bacterial cell density was determined by flow-cell cytometric (following Belzile et al. 2008).

Data analysis

We conducted an analysis of covariance (ANCOVA) to determine the effect that the explanatory variables – temperature, origin, and presence of a top predator – had on the response variables – bacterial density, respiration, and ammonium and phosphorus concentration. The analysis was performed on data from the last day of the experiment. The response variables were normalized using log transformation when appropriate. Temperature was a continuous variable and origin and predator presence were categorical. The comparison between the treatments on the last day of the experiment was possible because the replicate communities were standardized in each treatment at the beginning of

the experiment. All analyses were made with the software R (ver. 3.0.2; R Core Team) and residuals were checked with QQ-plots. Additional results that also include the 'mixed origin' treatment are presented in the Supplementary material Appendix 1, Table A2. The F statistic, degrees of freedom, and r^2 values for the ANCOVAs for each ecosystem function can be found in Table 1 (analysis conducted without the 'mixed community') and Supplementary material Appendix 1, Table A2 (analysis conducted with the 'mixed community').

The article's supporting data can be accessed at: <http://figshare.com/s/29338156a5a411e4a4f506ec4bbcf141>.

Results

Relationship between ecosystem function and temperature

The experimental results showed that temperature was the most important factor affecting all measured ecosystem functions, except for bacterial density, for which the origin of the community was the most important factor (p-values <0.001, Table 1; Supplementary material Appendix 1, Table A2; Fig. 2C, F). The ecosystem functions of phosphorus and ammonium production, and total respiration increased as temperature increased and this effect was independent of origin (e.g. Fig. 2F). Details concerning the among-replicate variation can be found in the Supplementary material Appendix 1, Table A3.

While the ANCOVA showed that there was a significant difference between origins (Table 1: origin F statistic $=264.53$, temperature F statistic $=58.88$, p-values <0.001), there was no relationship between the latitude of the origin and density. The communities obtained from Québec (northern part of the range) had the highest bacterial density and the ones obtained from Florida (southern part of the range) had the lowest, irrespective of the temperature at which they were incubated (Fig. 2C). However, we found no incremental change in density for communities located in the middle part of the range (Fig. 2C).

For total respiration and ammonium, the interaction between origin and temperature was also significant. However, the explanatory power (in regards to the F statistic) for the effect of temperature alone on respiration and ammonium was 18 and 48 times higher, respectively, than the interaction between origin and temperature. No interaction term was significant for phosphorus. For all measures of ecosystem functioning in all source populations, there was a positive relationship with temperature alone (Table 1, Supplementary material Appendix 1, Table A2).

Temperature strengthens top-down control

Presence and absence of the top predator had a significant interactive effect with temperature for all measures of ecosystem functions, except phosphorus (ANCOVA, all predator $\times$ temperature effects except phosphorus $p<0.05$, Table 1, Supplementary material Appendix 1, Table A2 and A3). The exception was the result for phosphorus, for which we did not observe a temperature dependence on the effect of the larvae (the predator $\times$ temperature interaction, F statistic $=0.95$, $p>0.05$, Table 1; Supplementary material Appendix 1, Table A2 and A3, analysis with 'mixed' community, F statistic $=0.8$, $p>0.05$). For bacterial density, respiration and ammonium concentration, we found that the top-down effects of the larvae increased with temperature (Fig. 3B–C). The relationship between the measures of ecosystem function and temperature-induced top-down control remained qualitatively the same in all origins (Supplementary material Appendix 1, Table A3). This result was also observed in the mixed community (Supplementary material Appendix 1, Table A3). We also observed a six-fold higher pupation rate in the warmest temperatures (53 pupation events in the Florida incubators in contrast to eight pupation events in the Québec incubator), which indicated an effect of temperature on larvae metabolic activity. The data therefore most strongly supported the synergistic effect of temperature and temperature-induced consumer-resource interactions (Fig. 3A). Note however the effect size of top-down control was much smaller than the effect of temperature (Table 1, Fig. 3), yet it is possible that this effect size would change if more mosquito top predators were added to the system.

Discussion

We integrated three distinct fields – ecosystem ecology, community ecology, and biogeography – to predict the effect that climate change will have on ecosystem functioning. Our experimental results show that temperature is the primary

Table 1. Summary of the ANCOVA tables for different ecosystem functions. Temperature was treated as a continuous variable. The 'origin' treatment includes the 5 original sites, without the 'mixed origin' treatment. The F statistic is reported for each function and treatment. Statistically significant treatments at $p<0.001$ are indicated by ***, $p=0.01$ by **, and at $p=0.05$ by *. Post-hoc Tukey's tests revealed significant differences between the mixture and other origins, but they were never found systematically larger or lower than all other origins over the different temperatures.

Treatment	DF	Bacterial density	Respiration	[Ammonium]	[Phosphorus]
Larvae	1	4.48*	3.41	18.39***	1.90
Origin	4	264.53***	22.12***	48.16***	12.33***
Temperature	1	58.88***	322.40***	113.30***	56.53***
Larvae × Origin	4	2.68*	0.76	2.19	0.77
Larvae × Temperature	1	9.27**	5.32*	11.58***	0.95
Origin × Temperature	4	0.85	6.68***	6.29***	0.41
Larvae × Origin × Temperature	4	0.74	1.19	2.80*	2.21
R^2		0.81	0.63	0.59	0.17

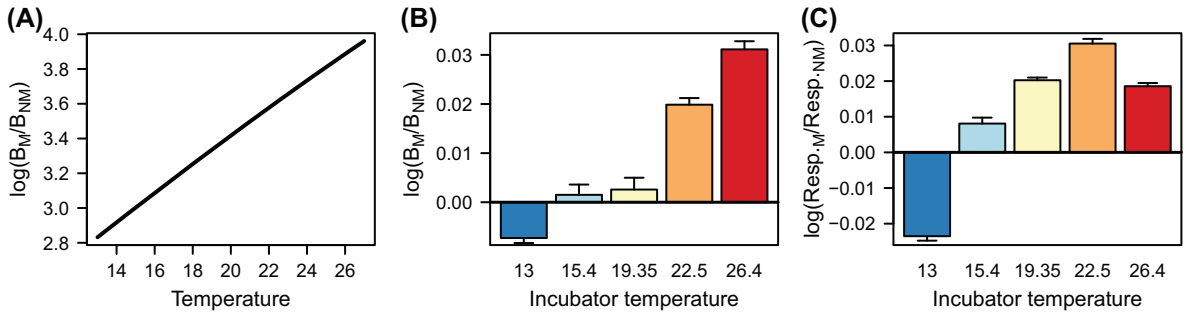

Figure 3. Impact of trophic control along the temperature gradient. Model prediction (A). Experimental results for (B) bacterial density and (C) total respiration (see Table 1 for ANCOVA results). The strength of trophic control is measured as the ratio of an ecosystem property in presence of the mosquito larvae (e.g. bacterial density, B_M) and in its absence (B_{NM}). Data from all five origins were pooled to produce this figure. The trophic control is stronger at the ends of the temperature gradient, with higher temperatures resulting in an increased functioning in the presence of the predator, and lower temperatures giving the opposite result.

driver of changes in ecosystem functioning, with warmer temperatures consistently increasing in ecosystem functioning. Trophic control also has a considerable effect on communities maintained at warmer temperatures. The response of this ecosystem to changes in temperature is therefore explained by the joint action of trophic structure and temperature. However, we found that the strength of the effect of temperature on metabolic rates generally surpasses its effect on predation rate (in agreement with Rall et al. 2012). Interestingly, the results from this study also show that the effect of origin on ecosystem functioning is significant, but the ability to decipher how communities from different origins will respond was not predictable according to their location along a latitudinal gradient. Instead, the effect of predation, albeit much weaker in comparison to the other effects, is correlated with latitude, in that communities in warmer temperatures were more affected by predation than communities in colder temperatures.

If the origin of a species was the most important factor driving ecosystem functioning (Fig. 2), then selection for the fittest species at a given temperature would consequently promote a community that is the most efficient at processing ecosystem functions at those temperatures. Our experimental results would have shown that the consumption rate for any particular bacterial community was maximal at the ambient temperature of that site and diminished as the community moved away from its ambient temperature. In terms of our experimental design, we thus would have seen a significant interaction between origin and temperature, and no a priori independent effects of temperature and origin. For the mixture of origins into a common species pool, we would have expected no specific relationship with temperature and consistently high functioning at all temperatures because the fittest species would systematically out-compete the others.

We found that our results are only weakly consistent with these predictions for the local adaptation hypothesis, as we did observe interactions between origin and temperature for some ecosystem functions (Table 1, Supplementary material Appendix 1, Table A3). However, in all cases, the effects of temperature are much stronger than the effect of species-sorting or adaptation (Table 1, Supplementary material Appendix 1, Table A3). These observations suggest that differences in functioning and response to warming is mostly explained by changes in metabolic rates. While we cannot rule out the action of local adaptation through species-level selection in our system, we can assume that its impact on ecosystem functioning was limited compared to other factors.

Our conclusion of a limited effect of local adaptation is not consistent with the current interpretation of metacommunity dynamics and biogeography, which are both strongly grounded on the Grinellian niche concept. The interpretation of our results must, however, take into account the characteristics of this system, in which the diversity of bacteria is high and thus may provide an insurance effect (Yachi and Loreau 1999). Given that the bacteria from any site must withstand large temperature fluctuations from Florida to Québec (e.g. 32°C to 7°C, respectively, in June), it is likely the microbial communities from each site contains a wide range of thermal resistance and could thus cope with warming (average yearly min./max. temperature according to WorldClim within the FL site: 5/32.7°C and QC site: $<$ 0/21.8°C). The hypothesis that the more diverse communities and the ones subjected to stronger temperature fluctuations have a buffered response to climate change does not have any empirical support so far. Testing it with microbial systems requires a significant amount of work, as it will require not only an exhaustive comparison of community composition with next generation sequencing (Poisot et al. 2013), but also an extensive assessment of the thermal niches of constituent strains for each origin with a particular focus on the rare species. Furthermore, over the course of the experiment, phenotypic plasticity likely played a role and even some components of the community may have evolved (the bacteria would have undergone several generations of selection for different temperatures). We believe, however, that the temperature gradient we imposed was large enough and the time scale of the experiment was short enough to minimize any insurance effect or phenotypic plasticity as potential explanatory mechanisms.

Metabolism has long been recognized as a key process responsible for energy flow in ecosystems. Our study is generally consistent with the metabolic theory in ecology, which proposes that basic metabolic rates of an organism can be predicted from temperature (Brown et al. 2004). When coupled to the scaling of energy flow across trophic levels

(Vasseur and McCann 2005), our study provides a framework to incorporate the effects of climate warming on the functioning of ecosystems (Yvon-Durocher et al. 2010). In addition, our experiment illustrates that the classical theories in food web and ecosystem ecology must remain tightly coupled in order to predict how species assemblages will respond to global changes (Lavergne et al. 2010), because these theories could interact as 'biotic multipliers of climate change', causing cascading effects in communities (Zarnetske et al. 2012). A more extensive theory of the effect of temperature on ecosystem functioning will be required to take into account a wider range of responses to temperature and of food-web configurations. It is also necessary to begin to couple this theory with a more in-depth examination of the effect of community composition on ecosystem functioning. For microbial systems, in particular, the use of molecular techniques will allow a better understanding of how changes in species composition due to warming, local adaptation, and predation may alter ecosystem functioning.

To date, there are many studies focusing on how temperature influences ecosystem functioning (Yvon-Durocher et al. 2012) and biotic interactions (Gilman et al. 2010), but most of these studies are individual cases examining just one dimension of climate change. There is a need to integrate these concepts, which will allow for the study of synergistic effects of climate and biotic interactions on ecosystem functioning. The need for such a synthesis is particularly exemplified by our finding that trophic control of ecosystem function varies with temperatures. Accomplishing this objective will help ecologists to explore a wide variety of questions, ranging from the most fundamental (explaining the worldwide repartition of biomass and productivity, Huston and Wolverton 2009) to the most pressing conservation-oriented issues (Pereira et al. 2010).

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Supplementary material (Appendix ECOG-01748 at <www.ecography.org/appendix/ecog-01748>). Appendix 1.