A general temperature dependence for biological nitrogen fixation across systems and levels of organization

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Abstract

Biological nitrogen fixation (BNF) provides half of global new nitrogen annually and plays an important role in biodiversity patterns and global biological carbon uptake processes. BNF rates accelerate with warming, with known implications for ecological functioning, yet the strength of this temperature sensitivity and its context dependence are not well understood. Here we synthesize 70 controlled experimental tests of the acute temperature dependence of nitrogen fixation rates and demonstrate that BNF rates accelerate with temperature in a remarkably consistent manner across biological systems. BNF temperature dependence is also consistent across levels of biological organization from enzyme to community, mirroring scaling of metabolic temperature dependence across levels of organization for respiration, photosynthesis and methane production. This analysis provides the first evidence of general, scalable effects of temperature on nitrogen fixation rates, which can be used to better predict shifts in coupled carbon-nitrogen systems with global change.

Introduction

Biological nitrogen fixation (BNF) converts inert dinitrogen (N_2) to bioavailable ammonium (NH_4^+), and is the only metabolic link between the vast stores of atmospheric nitrogen and the

biosphere. Nitrogen fixers, hereafter diazotrophs, occur across a vast diversity of ecosystems (Figure 1) and provide 50% of annual "new" nitrogen, globally [1]. This nitrogen plays an important role in biodiversity patterns and global biological carbon uptake processes by facilitating primary production in low and variable N environments [2–5]. The relationship between BNF and oxygenic photosynthesis is nearly as old as photosynthesis itself [6, 7]; close association with nitrogen fixation is common across the tree of photosynthetic life, including cyanobacteria, protists, lichens, and lower and higher plants [8, 9]. As climate warming accelerates metabolic demand across all systems, it is not clear how N production from BNF responds to warming, and whether this response is sufficient to support photosynthesis in warming ecosystems. While recent global change models acknowledge that nutrient limitation will be a key driver of carbon fixation rates with warming [10], inclusion of direct effects of temperature on BNF rates are rarely included in coupled C-N models [11]. A deeper understanding of these effects is needed to predict changes in ecological functioning with warming [12].

It has long been understood that, because breaking the triple bond in a dinitrogen molecule is an energy intensive process, nitrogen fixation rates are highly temperature sensitive [13, 14]. The strong temperature sensitivity of BNF has been invoked to suggest that BNF is a more viable nitrogen acquisition strategy at higher temperatures [2]), and in turn to explain several broadly important ecological phenomena, including spatio-temporal variation in the occurrence of cyanobacteria blooms [15] and the geographic distribution of nitrogen fixation in marine pelagic [16–18] and terrestrial, tree-associated nitrogen-fixation [2, 3, 19] systems. While these explanations have proven useful for describing ecological processes in diverse systems, the lack of a common mechanistic understanding has led to disparate predictions for how a warming climate affects the productivity of nitrogen fixing systems, with contrasting implications for coupled N- and C-budgets at larger scales [2, 19–21]. In order to resolve these conflicting predictions, we first need to understand whether one general model of temperature effects on BNF rate is appropriate across ecological systems.

Across its broad ecological and taxonomic domain, BNF is mediated by a single enzyme, nitrogenase, with minimal structural and biochemical variation among taxa [22–25]. From this, a mechanistic hypothesis is that the effect of temperature on the kinetics of this enzyme also varies minimally across organisms employing this common biochemistry [26]. Further, if enzyme-level BNF rates constrain BNF rates at higher levels of biological organization, as suggested by metabolic scaling theory cf. [27], we would expect to see similar temperature effects on BNF

rates at organism, population, and ecosystem levels [27, 28]. Indeed, general effects of temperature on metabolic rate across biological systems and levels of organization have been observed for other highly conserved metabolic pathways, including aerobic respiration [29, 30], photosynthesis [31, 32], and methanogenesis [28]. This metabolic scaling approach provides an efficient first order assessment of whether simple models of temperature effects on enzyme (here, nitrogenase) activity can describe broad-scale temperature effects on metabolic (here, BNF) rates. If, however, large ecological and evolutionary differences among nitrogen-fixing systems alter the temperature-enzyme activity relationship, or downstream processes depend on BNF, then more complex models may be needed to predict changes in BNF-sourced N supply with warming.

Here, we conducted the most comprehensive data synthesis to date of observed BNF temperature responses to assess patterns in the temperature dependence of BNF across ecosystems and levels of biological organization. We draw from metabolic scaling theory to formulate and test three hypotheses. First, we test a general temperature dependence hypothesis that the temperature response of nitrogen fixation should be similar across ecological systems, because it is underpinned by a kinetic response of the enzyme nitrogenase in each of these systems. However, because energy and resource dynamics underlying observed temperature responses can vary with ecological and evolutionary context, we tested the general temperature dependence hypothesis against an alternative systematic temperature dependence hypothesis, that the temperature dependence of BNF varies with one or more of the following: nitrogen fixer taxonomy, habitat type, photosynthetic association, and thermal acclimation history. We estimated the effect of temperature on BNF-rate independently at enzyme, population, and community levels by using linear mixed effects models to fit the Boltzmann-Arrhenius function to datasets at each level of organization. Then, we then compared the general and variable temperature dependence hypotheses using AICc-based model selection ([28]). Thus, for the purposes of this study, general versus variable temperature dependence are considered mutually exclusive, where the temperature dependence of BNF at each level of organization is either consistent across studies or variable with some ecological or evolutionary factor(s) identified in the dataset (Table S1). Finally, we utilized model estimates from the enzyme-, population-, and community-level analyses to test a third hypothesis, that a common enzyme-level BNF temperature-dependence constrains BNF temperature responses at population and community levels. This metabolic scaling hypothesis predicts that temperature dependencies at these

higher levels of organization will mirror the enzyme-level response. Testing these hypotheses independently identifies potential drivers of deviation from enzyme-level constraints as systems become more complex. In doing so, we determine whether simple scaling relationships can be used to describe temperature-driven change in nitrogen fixing systems.

Results

An analysis of BNF temperature responses from 70 controlled experiments within 27 studies revealed a consistent response of BNF to temperature of 1.03 eV from enzyme to community levels (Table S5, Figure 2, raw data shown in Figure S1). We found stronger support for a general temperature dependence across systems than for variable temperature dependencies at population and community levels, and mixed support at the enzyme level (Table 1). The enzyme-level BNF temperature dependence estimate was included within 95% confidence intervals for all higher-level temperature dependence estimates, in support of the metabolic scaling hypothesis (Fig. 3). We compared model fits for linear and nonlinear models and found that they produced nearly identical temperature dependence estimates (Figure S2). For parsimony, we present results from analyses of linear models.

Across the enzyme-level dataset (n = 4 temperature responses), the estimated BNF temperature dependence was 1.09eV, 95% CI: 0.71 to 1.47 eV(Figure 2A). Model comparison between a general temperature dependence model and a variable temperature dependence model revealed equivocal evidence for a single temperature dependence across these studies: the best model included only a main effect of temperature on BNF rate, but the second best model (Δ AICc = 1.39) included variation in the temperature effect with nitrogenase source-species (Table S2). In the variable temperature dependence model, there was a substantial difference in the predicted BNF temperature dependence between the two nitrogenase source species represented in the dataset: nitrogenase isolated from *A. vinelandii* (n = 2) had an E_a of 1.32 eV, 95% CI: 0.95 to 1.69 eVand nitrogenase isolated from *K. pneumoniae* (n = 2) has an E_a of 0.75, 95% CI : 0.21 to 1.29 eV(Figure S3).

Population-level analysis (n = 39 temperature-responses) revealed a BNF temperature dependence of 1.02 eV with 95% CI: 0.61 to 1.42 eV, similar to that of the nitrogenase enzyme (Figure 2B). The general temperature dependence model outperformed models that allowed temperature main effects to vary systematically with habitat, diazotroph order, or thermal acclimation

history (Table S3). Community-level analysis (n= 27 temperature-responses) revealed a BNF temperature dependence of 1.15 eV with 95% CI: 0.67 to 1.63 eV, similar to those at the population- and enzyme-levels (Figure 2C). Here, model selection indicated stronger support for the general temperature dependence model than for a model including temperature main effects to vary with photosynthetic association (Table S4). Exceptionally strong temperature responses in population- and community-level datasets (6 in each dataset; Figure 2B and C insets) are likely attributable to a study-level random effect, because the systems included in these rates were represented elsewhere in the data, and all of the extreme temperature sensitivity values were reported in one study in the population dataset, and mostly one in the community dataset.

A cross-scale model comparison revealed that a model with a single temperature coefficient across all three levels of organization outperformed a model that fit separate slopes at each level (Table S5), and produced a BNF temperature dependence estimate of 1.03 ± 0.13 eV. Together with the confidence interval analysis (Figure 3), these two lines of evidence provide strong support for the metabolic scaling hypothesis.

Discussion

Ecological models using metabolic scaling theory have provided parsimonious and simple explanations for macroecological patterns from biogeographic variation in ocean net carbon metabolism [32] to biodiversity [33] to methane production [28]. Our analysis has provided evidence that the rate of new nitrogen production through biological nitrogen fixation (BNF) meets the criteria for inclusion in metabolic scaling theories. We estimated an enzyme-level BNF temperature dependence of 1.09 eV that emerged at the population and community levels, consistent with the hypothesis that the temperature dependence of the enzyme-level metabolic system scales to higher levels of organization. This estimate is consistent with previous work on ecosystem-level terrestrial nitrogen fixation (approximately 1.07 eV; [2]) and substantially lower than the widely used enzyme-level estimate (2.15 eV; [13], see Supplemental Information for further consideration). Additionally, general temperature dependencies emerged at the population or community level. Together, these results provide evidence for a strong and consistent temperature dependence of nitrogen fixation across ecosystems and levels of biological organization.

We tested for a common enzyme-level temperature dependence of BNF to understand whether a thermal constraint on metabolic rate could be observed at population and community levels [27, 29], and to contribute a rare data synthesis at this basal level of organization (but see [26]). While an enzyme with a consistent structure and biochemistry should theoretically have a characteristic temperature dependence, we found weak evidence that the temperature dependence of nitrogenase activity may vary depending on source species, *Azotobacter vinelandii* and *Klebsiella pneumoniae*(Table S2). This is difference is surprising, because, while these two species can utilize slightly different variants of nitrogenase [34], there is evidence that these nitrogenases are nearly perfectly cross-compatible [22, 35]. With only two taxa, we are limited in the capacity to extend this inference to broader groups; a comprehensive treatment of enzyme-level temperature effects requires data from across all nitrogenase groups [23, 36].

From possible enzyme-level differences in BNF temperature response across species, we might expect to observe systematic effects of species identity on temperature dependence or much wider confidence intervals around the estimate in the population and community level datasets, which contain many taxa across diverse Orders (Table 1). Instead we found that BNF temperature dependencies at higher levels of organization showed similar temperature dependence to the mean enzyme-level temperature dependence. Further, population-level temperature dependencies (N = 39) did not vary with diazotroph taxonomy, which spanned seven Orders. This suggests that, despite our small dataset at the enzyme level, we may have captured a central tendency in enzyme-level BNF temperature dependence. Finally, our ability to recover similar BNF temperature dependence estimates across three levels of organization, and terrestrial and aquatic systems, suggests that the nitrogenase enzyme predictably constrains BNF rates across levels of biological organization.

Identifying systematic differences in how BNF-sourced nitrogen supply and photosynthetic nitrogen demand respond to warming is important for anticipating shifts in C metabolism with warming. Across ecosystems and levels of organization, the temperature dependence of BNF rate is nearly always stronger than that of photosynthesis (0.3 - 0.5 eV, [37–39]). In other metabolic supply and demand systems, thermal asymmetries, or differences in temperature dependence between metabolic processes, underlie change at the ecosystem level. For example, a thermal asymmetry between aerobic respiration and oxygenic photosynthesis, which are metabolically linked in ecosystems by carbon and oxygen fluxes, has been used to explain shifts in community-level species interaction strengths, and ecosystem NPP with warming

[31, 32, 40]. This is consistent with a generalized prediction that less temperature sensitive metabolism in a resource exchange network will constrain the rate of more temperature sensitive metabolisms with warming [39, 41]. The BNF-photosynthesis thermal asymmetry differs from the respiration-photosynthesis thermal asymmetry in that the process traditionally viewed as a resource-supply process, BNF, has a stronger temperature dependence than the resource-demand process, photosynthesis. How this type of thermal asymmetry will affect C fluxes with warming is unclear.

A thermal asymmetry between BNF and photosynthesis has led to contrasting predictions about net effects of warming on BNF-sourced nitrogen supply to photosynthesis. One possible outcome is that nitrogen supply via BNF will exceed nitrogen demand via photosynthesis at warmer temperatures. This mechanism has been invoked to explain larger than expected increases in stream primary production with warming [20, 42], and may maintain productivity when temperatures exceed the range of non nitrogen-fixing algae [43]. Alternative outcomes of the BNF-photosynthesis thermal asymmetry involve reshaping carbon-nitrogen exchange at high temperatures through energy-limitation of N-fixation [2] or decoupling N and C metabolisms [21]. These outcomes would be broadly consistent with the thermal resource constraint dynamics that have been observed in the photosynthesis-respiration thermal asymmetry [32]. Interestingly, observations of nitrogen fixation boosting photosynthetic production with warming have occurred primarily in aquatic systems [20, 42], where C- and N-fixation typically occur within a single cell, while observations of carbon-limitation of nitrogen fixation have occurred primarily in terrestrial systems [2, 21], where C- and N-fixation occur in different organisms. In the latter scenario, N supply and N demand are generated by organisms with substantial differences in body mass and generation time, and must be transported across many membranes and sometimes substantial distances (e.g., from tree root systems to leaves). Whether these different types of metabolic resource networks respond similarly to warming is an open question with important consequences for ecological functioning under global change.

Despite the importance of BNF to the global nitrogen cycle and to local carbon fixation, two recent studies revealed that only two of fourteen Earth systems models used by the IPCC's assessment process include direct effects of temperature on BNF rates [11, 44]. The exceptions are phenomenological models specific to terrestrial symbiotic N-fixing systems. Our tests of the general versus systematically variable temperature dependence hypotheses and metabolic scaling hypothesis link mechanistic temperature effects with BNF rates in complex systems and

suggest generality across a much larger breadth of systems. Our estimate for the temperature dependence of BNF includes data from aquatic and terrestrial habitats, symbiotic and free-living organisms, at least seven diazotroph orders (taxonomy not reported for community-level studies), and a broad geographic distribution (Figure 1). At enzyme and population levels, our dataset represents two major taxonomic groups of nitrogen fixers, the proteobacteria and cyanobacteria, which comprise a substantial portion of diazotrophic diversity [23, 36]. Thus, our analysis contributes a novel and robust estimate of BNF temperature dependence appropriate for use in macroecological models, but system-specific models may require data from groups not represented here.

The response of photosynthetic carbon flux to warming in ecosystems depends on access to resources [10, 45, 46]. Here, we present evidence that biological nitrogen fixation rates are likely to increase faster than photosynthetic rates as systems warm. Among contradictory predictions for how BNF will play into global C budgets with warming, we suggest that paired measures of photosynthetic and BNF temperature responses in controlled experiments (such as [21]) and in natural contexts are needed to identify key resource constraint processes across thermal gradients. This treatment of temperature-resource interaction as a continuous and dynamic ecological process will be central to understanding how realized warming responses in ecosystems emerge from fundamental ones.

Methods

Data aggregation

We tested the three hypotheses using data from published studies that presented BNF rate measurements over an experimental temperature gradient. We identified papers in Web of Science using the search terms "nitrogen fix*" and "temperature." We screened 5143 papers for broad relevance, first by title, then by abstract using the revtools package in R ([47], R version 4.0.2). We conducted a full text review on all papers that indicated in the abstract that nitrogen fixation rate measurements were reported across multiple temperatures. Criteria for data inclusion were: that all BNF rate measurements were mass-corrected (e.g., units N fixed per unit mass per unit time), that a set of rate measurements were made over a minimum of four temperatures below the thermal optimum (see Fig. S4), and that temperature

measurements were instantaneous (i.e., not averaged over time or space). We requested raw data from authors and extracted data from papers using WebPlotDigitizer [48] when author-sourced data were unavailable. We categorized each BNF temperature response by the level of biological organization (i.e., enzyme, population, or community) at which data were collected, to test our scaling hypothesis. Enzyme-level studies were identified by the isolation of nitrogenase enzyme for empirical estimation of BNF rate. Studies were categorized as population-level if a single nitrogen-fixing taxon was identified in the study and categorized. Studies were categorized as community-level if the nitrogen fixers were described in the research article as multi-species or if nitrogen fixation was measured on an unmanipulated sample of a natural substrate, such as a soil core or segment of a log. The full dataset included 70 unique BNF thermal responses, across 27 studies (see GitHub repository).

Model fitting

To test our general temperature dependence hypothesis and alternative variable temperature dependence hypothesis, we fit thermal response models to each set of BNF rate measurements across a temperature gradient (hereafter BNF temperature-response), at each level of biological organization. The general temperature dependence hypothesis was formulated using the linearized Arrhenius model [27]:

$$\ln\left(\frac{B}{M}\right) = E_a\left(-\frac{1}{kT}\right) + \ln(B_0) \tag{1}$$

where the biomass-corrected nitrogen fixation rate, $\frac{B}{M}$ (e.g. g N fixed / hr /g biomass), is related linearly to inverse temperature, $1/(kT)$ (K), via Boltzmann's constant, k (eV/K), the temperature dependence parameter, E_a (eV), and the model intercept, B_0 (units N fixed per unit mass per unit time). The parameter, E_a, represents the slope of the log rate-temperature relationship and is the focal parameter of our analysis.

We estimated the value of E_a for each BNF temperature-response using linear mixed effects models that took the following form:

$$\ln B_{i,j,m}(T) = \left(E_a + \epsilon_{E_a,i,j,m}\right)\left(\frac{1}{k\,\overline{T}} - \frac{1}{k\,T}\right) + \left(\ln B_0(\overline{T}) + \epsilon_{B_0,i,j,m}\right), \tag{2}$$

where $B_{i,j,m}$ is the natural log of biological nitrogen fixation rate at temperature, T(K), for some observation, i, in each experimental unit, j, in study, m. Each experimental unit corresponds to a set of BNF rate measurements made on replicate enzymes, populations or communities for which all conditions were held constant except for temperature, referred to as a 'temperature response' in the main text. $\overline{E_a}$ (in eV) captures the temperature dependence estimated over all experimental units within the level of organization being analyzed. We centered temperature data using the mean temperature across all observations at each level of organization, $\overline{T}$ (K) at each level of organization. $\ln B_0(\overline{T})$ represents an average of BNF rate observations at mean temperature, $\overline{T}$. The model allowed for variation in E_a among experimental units and among studies, and modeled this variation using a random effect $\epsilon_{E_a(j,m)}$ with a normal distribution with a mean 0 centered on $\overline{E_a}$ (following [28, 49]. We also assumed variation among experimental units and studies in the intercept term $\ln B_0(\overline{T})$, reflecting variation in system-level BNF rates, experimental conditions and units in which BNF rates were reported.

To test the general temperature dependence hypothesis against the variable temperature dependence hypotheses, we compared (Eq. 2) to expanded models (e.g. Equation S1) with temperature interaction terms for ecological and evolutionary factors expressed as fixed effects (Table S1; see Supplemental Information). The factors we tested in our main analysis were habitat, thermal acclimation history, and diazotroph Order (Table 1. While we expected that other traits such as oxygen accomodation strategy (heterocysts, temporal C/N separation, or none) or symbiosis status (symbiotic or free-living) might be additional sources of systematic variation, all variation among taxa in symbiosis vs free-living life histories and in oxygen accommodation strategy was captured by Order, indicating complete phylogenetic co-variation among these factors in our data (Figure S5). At the community level, insufficient data across habitat types precluded us from testing habitat as a source of variation (Figure S6. All models included random effects of BNF temperature response nested within study on model slope and intercept (e.g. Equation S1) and were fit using the nlme package in R [50].

To facilitate linear model fitting across studies with different temperature ranges, some of which exceeded the thermal optimum, we analyzed only the increasing portion of each temperature response (Fig. S4). Metabolic temperature responses are generally non-linear and unimodal, and this shape can have consequences for the estimation of temperature dependence parameter, E_a [51, 52]. Fitting unimodal models to accurately described both the increase in rates at low temperatures and the decline in rates at high temperatures requires more and higher resolution data

than fitting the linearized Arrhenius function. Reducing our dataset to only those that could accommodate unimodal model fitting (41 out of our 70 temperature-responses) would have greatly reduced statistical power. However, to test for effects of curvature in BNF-temperature responses on estimates of E_a, we conducted a second analysis, where we fit quadratic models to each temperature response and transformed these model parameters to estimate E_a following Pawar *et al.* [51] Eq. 10. We found that estimates of BNF temperature dependence were largely consistent across the two methodologies (Fig. S2). We proceeded with the linearized Arrhenius model for hypothesis testing, as this model has wide use in metabolic scaling theory and the temperature-dependence term, E_a, is anchored in first principles of temperature effects on enzyme kinetics. We did not standardize rate units across studies and comparisons of model intercepts were not relevant to our hypotheses.

Model selection and inference

To determine whether a general temperature dependence or variable temperature dependence better described BNF temperature responses, we used small-sample-corrected AIC (AICc)-based model comparison with a threshold ΔAICc value of 2 [53] executed in the R package MuMIn [54]. We used the temperature terms retained in the best model to make inferences about the two alternative hypotheses: if no temperature interaction terms were included in the best model, we inferred support for a general temperature dependence; if at least one temperature interaction term was included in the best model, we rejected the general temperature dependence hypothesis [55, 56]. If the best model contained a temperature interaction term(s), we compared that model against a model that allowed only intercept to vary with factor(s) in the interaction term(s). We first fit models using maximum likelihood estimation to facilitate comparison of models with different fixed effects structures, then refit the best model using restricted maximum likelihood estimation to estimate model coefficients [57]. We conducted this model comparison analysis separately at each level of organization.

To test our metabolic scaling hypothesis, we took a two-pronged approach: first, we compared the 95% confidence intervals around model-estimated temperature dependencies at population and ecosystem levels with that at the enzyme level; second, we employed a cross-level model comparison approach to test whether a single temperature dependence term (E_a) across the whole dataset described the temperature dependence of N-fixation within a level of organization better than separate temperature dependence terms at each level. For each test, respectively, we

inferred evidence consistent with metabolic scaling if confidence intervals included the enzyme-level estimate, and if the best model included only a temperature term and no temperature by level of organization interaction. All analyses were conducted using R version 4.3.2.

Author Contributions

KED and MIO conceptualized the study. KED and MAS aggregated study data. KED analysed the data and wrote the manuscript. KED and MAS created figures and all authors contributed to revising the manuscript. MIO supervised the study.

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Data Availability

All data and code used to generate these results will be made public at the time of manuscript publication. In the meantime, please reach out the corresponding author with inquiries.

Tables and Figures

Table 1: Distribution of temperature-responses across ecological and evolutionary factors tested in our main analysis.

Level of organization (n)	**Factor**[†]	**Level**	**No. of temperature-responses**
Enzyme (4)	Nitrogenase source species	*Azotobacter vinelandii*	2
		Klebsiella pneumoniae	2
Population[*](39)	Habitat	Aquatic	18
		Terrestrial	21
	Diazotroph order	Nostocales	18
		Oscillatoriales	7
		Hyphomicrobiales	7
		Chroococcales	3
		Actinomycetales	2
		Rhodospirillales	1
		Pseudomonodales	1
	Thermal acclimation history	Acclimated	14
		Not acclimated	25
Community[*](27)	Photosynthetic association	yes	10
		no	17

[†] See Table S1 for definitions of each factor and level.
[*] See supplementary information for overlap between these factors at the population (Figure S5) and community (Figure S6) levels.

Figure 1: Nitrogen fixation is a diverse and widespread metabolic pathway. A) Protein structure diagram for the enzyme nitrogenase, which occurs only in prokaryotes, and underpins biological nitrogen fixation across all diazotrophic organisms. B) Some of the diverse ecosystems where nitrogen fixation occurs, including an antarctic ice-free plateau, coral reef, sphagnum bog, freshwater pond, lichen, soybean crop field, alder grove, seagrass meadow, and prairie grassland. C) Geographical distribution of nitrogen fixer observations represented in this study. Data points are plotted over the location of *in situ* experiments, the location of the original source site in the case of laboratory experiments using isolated strains (filled circles), or the location of the corresponding author's research institution if other information on source location was not available (open circles). Image credits: Nitrogenase enzyme structure - RSPDB M1N1; antarctic plateau - Dr. Amit Dharwadkar; sphagnum bog - Wikimedia commons; soybean field - Wikimedia commons; freshwater pond - Jason Laurich; lichen - Andrew Simon; prairie grassland - Wikimedia commons

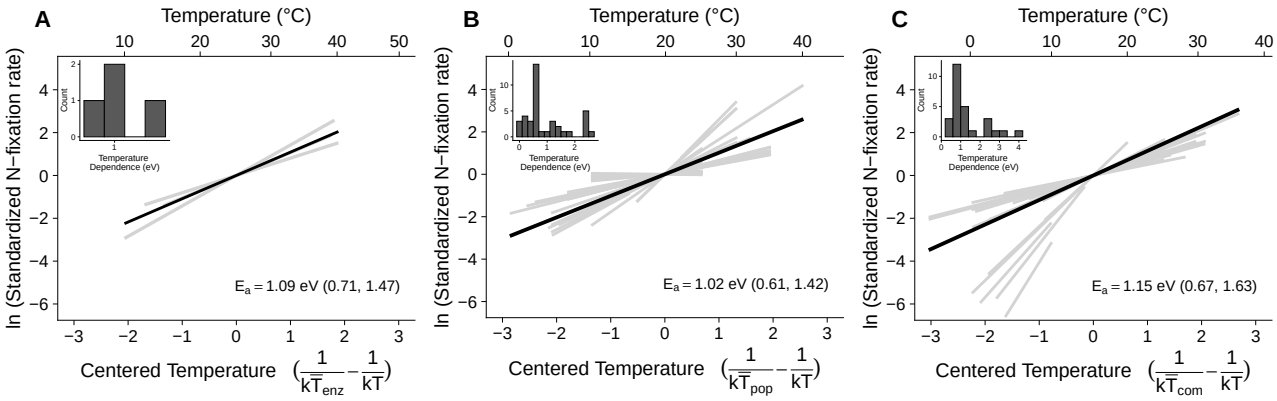

Figure 2: General temperature dependence of BNF across three levels of organization, A) enzymes, B) populations, and C) communities, based on hierarchical mixed effects model analysis. Model-predicted BNF rate-temperature relationships (black lines) and raw temperature response data (grey). Nitrogen fixation rates are standardized to the predicted rate at the mean temperature for the level of organization. Insets show the distribution of these model fits, including random effects, at each level of organization. Model estimates are displayed over raw data in Figure S1.

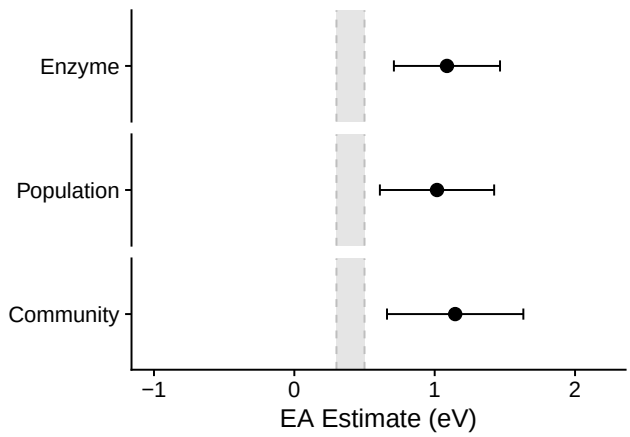

Figure 3: Metabolic scaling of BNF temperature dependence. Enzyme, population, and community levels BNF temperature dependence estimates from the top-performing models are shown with temperature dependence estimates and 95% confidence intervals are displayed. The grey rectangles display the range of temperature dependence estimates commonly used for photosynthesis, 0.3-0.5 eV.

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