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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection.
Data analysis	All analyses were conducted in R (version 3.5.2), using the packages "MASS" (version 7.3-51.1), "plantecophys" (version 1.4.4), and "bbmle" (version 1.0.23.1). R code is publicly available on GitHub (https://github.com/tbytnero/Bytnerowicz-Akana-Griffin-Menge-N-fix-Temp). This repository has a Zenodo DOI (https://doi.org/10.5281/zenodo.5764790).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

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Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	Four N-fixing tree species that represent tropical or temperate origins and rhizobial or actinorhizal symbiotic associations were grown in plant growth chambers at three growing temperature regimes spanning a range of 10 deg. C. We measured the temperature responses of whole-symbiosis N fixation and leaf-level net photosynthesis, Vcmax, Jmax, and respiration and the interaction between measurement temperature and length of exposure on N fixation.
Research sample	N-fixing tree species were Morella cerifera, Alnus rubra, Gliricidia sepium, and Robinia pseudoacacia and were chosen to represent tropical, temperate, rhizobial, and actinorhizal origins and symbioses. Alnus and Robinia are the most common temperate N-fixing tree species in North America and are also common genera in Europe. N-fixing tree species in the tropics are much more diverse, however, Gliricidia and Morella are relatively common in the neotropics. Tree species were grown from seeds purchased online and were inoculated with field-collected symbiotic bacteria (Frankia or Rhizobia). Seeds originated from Louisiana, Washington, India, and Germany and nodules from Florida, Oregon, Florida, and New York for Morella, Alnus, Gliricidia, and Robinia, respectively. For further details see Supplementary Information.
Sampling strategy	Temperature response measurements were replicated across three separate individual seedlings each. By fitting curves across all three growing temperatures, N fixation, photosynthesis, and respiration temperature responses were fit to nine individuals for each species. Power analysis with preliminary points suggested that these sample sizes were sufficient.
Data collection	Data were collected by T.A.B. and P.R.A. N fixation measurements were made following Bytnerowicz et al. (2019) Methods in Ecology and Evolution. This involved making whole-plant, non-destructive, and continuous measurements of acetylene reduction at 2% acetylene. The substrate-saturated rate of acetylene reduction was calculated from this by measuring how the acetylene reduction rate changed with acetylene concentrations and how this varied across species, growing temperatures, and measurement temperatures. Substrate-saturated acetylene reduction rates were converted into N fixation rates by making paired nodule-level incubations of acetylene reduction and 15N2 gas across species, growing temperatures, and measurement temperatures. The interaction between temperature and length of exposure on N fixation was determined with continuous 6-hr measurements at a series of measurement temperatures. Leaf-level carbon exchange measurements were made with a LI-COR 6800 photosynthesis system.
Timing and spatial scale	Our experiment was conducted between December 2016 and March 2020. This began with the germination and inoculation of seedlings in December 2016. Data collection started February 2018 and was concluded March 2020. The experiment took place in a lab setting at Lamont-Doherty Earth Observatory (Palisades, NY) but used species with both temperate and tropical origins.
Data exclusions	Prior to analysis, N fixation temperature response data were visually inspected and artifacts (anomalously low or high ethylene points) were removed (i.e., 2 % of N fixation temperature response data). Where the half-saturation constant (Km) was measured for calculating substrate-saturated rates of acetylene reduction (Vmax(t)), all measurements at 40 deg. C, with the exception of Morella from the warmest growing temperature, were excluded due to declining N fixation rates during the course of measurement. A275, Vcmax, and Jmax calculated from A-Ci curves that did not meet our criteria were removed. Conversion factors between acetylene reduction and N fixation which were more than 3 units below or above the theoretical value of 4 were considered outliers and were removed prior to analysis. These outliers typically came from cases with very low values of Vmax and 15N2 incorporation, which meant that conversion factor ratios were highly sensitive to measurement errors. Where the effect of prolonged exposure to temperature was determined (temporal-decline model), three individuals were removed due to "b" values that were considered outliers. Criteria for exclusion were pre-established for the Km and carbon exchange measurements but not for the N fixation temperature response artifacts, conversion factor, and temporal-decline model. See Supplementary Information for further details.
Reproducibility	We replicated each sampling unit (species grown at a growing temperature regime) three separate times, as described in the text and shown in Fig. 1. The replicates were as independent as is possible given the conditions. (I.e., they were separate individual plants, but were from the same seed source, and were inoculated with the same mix of bacteria.) We have provided sufficiently detailed methods in the Main Text and in the Supplementary Information to allow others to reproduce the study.
Randomization	We randomly chose three individuals per treatment from each species and growth chamber.
Blinding	Blinding was not possible in our setup. In this context, blinding would mean not knowing the species identity of the plant or its growth temperature regime. However, the species look different, so we can tell which species is which, and we know which growth chamber we took each individual from, so the measurements could not be blinded. That said, the instruments making the measurements (the Picarro and the Licor) did not know which species or which growing temperature each plant was from, so the potential for bias due to the lack of blinding was negligible.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging