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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

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

Commercial software used to control and collect data form the following instruments were used as follows;
• Online volatile organic compound concentrations were collected the commercial PTR-MS software for quadrupole mass spectrometers (PTR-MS Control software, Ionicon Analytic)
• Online 12CO2, 13CO2 (isotopic CRDS) and high precision O2 CRDS concentrations data were collected using the commercial Picarro Surveyor software (Picarro Inc.)
• Online leaf photosynthesis, transpiration, and stomatal conductance data as well as controlled environmental variables were collected using Bluestem OS. Version 2.1.13 on a Li6800 portable photosynthesis system (Licor, Inc.)
• Branch level fluxes of leaf photosynthesis and transpiration were acquired using a Li7000 under computer control using the Li-7000 Control software (Licor, Inc.)
• Extracted leaf metabolite data using LC-MS were collected using Thermo Xcalibur software.
• Verification of leaf methanol 13C-labelign by GC-MS was collected using Masshunter version 11.1 (Agilent)
• Data for analysis of methyl ester 13C-labelling patterns of isolated whole leaf cell walls by GC-C-IRMS was collected using Isodat (Thermo)
• Using STRING-DB V12.0 (Search Tool for Retrieval of Interacting Genes/Proteins DataBase), orthogroups of proteins of the seven genes in the C1 pathway were identified across all organisms available in STRING-DB.
• To construct a phylogenetic tree, all protein sequences from the methionine synthase (MS) orthogroup were extracted from the STRING-DB and manually curated to filter sequences specific to two diatoms, two green algae, one moss and 15 land plants (dicots and monocots). Two cyanobacterial MS sequences were extracted from Cyanobase. In total, 76 sequences from 22 species were used to construct the tree (see supplemental Table S1).
• Environmental data (air temperature) during field studies were collected using Omega Data Logger Software (ODLS): A software platform for configuring, downloading, and analyzing data from Omega data loggers, including the OM-CP-OCTTCTEMP-A2 used here.

	• Sap Flow Tool version 3.1.4 (ICT International) was used to configure SFM1 sap flow sensors as well as download data.
Data analysis	• All leaf and branch gas exchange time series data were analyzed using Igor Pro software (version 8.0). • For leaf metabolite analysis, Raw LC-MS files were converted to MZML using ProteoWizard MSConvert 3.0.2126582 or ThermoRawFileParser version 1.4.2. Data were evaluated and peak heights for methionine and singly 13C-labeleled methionine were extracted using MZmine 2.083. MS2 spectra were extracted using the RaMS package version 1.3.184 and plotted in base R 4.3.085; vector-based PDFs were generated using Cairo 1.6. • To construct a phylogenetic tree for methionine synthase (MS), a multiple sequence alignment (MSA) of the selected protein sequences was performed using the msa() function with the ClustalW method in R version 4.2.2. A maximum likelihood (ML) tree was generated using the phangorn package in R. Based on the MSA, various models were tested for the estimation of likelihood to the provided sequences with the modelTest() function and the best available model was selected to construct a ML tree using the pml_bb() function. To improve confidence, the tree was bootstrapped a 1,000 times using the default ultrafast bootstrapping in the phangorn() package. The tree was plotted using the ggtree package in R. The sequences were checked for localization within the chloroplast by investigating the presence of transit peptide in TargetP and prediction of plastid localization using DeepLoc2.0 (0.9 cutoff). Sequences with either transit peptides or predicted plastid localization were manually annotated on the tree.

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

A data availability statement has been included as follows: All data needed to evaluate the conclusions in this paper including the source data of every figure and supplementary figure is available for download as a supplementary file.
• All raw LC-MS/MS data are available in the MassIVE repository (https://doi.org/doi:10.25345/C58S4K08B, User: MSV000093760_reviewer, password: 13C1Methionine).
• See Supplementary Information section for a description of all supplementary files available for download which includes experimental data from every figure and supplementary figure.

Supplementary Information
Figures and Supplementary Figures data: Data set for all data-based figures including Figure 1a, Figure 1b, Figure 2, Figure 3, Figure 7a, Figure 7b, and supplementary Figures S1, S2, S3, S4, S5, S6, S7, S8, S9, S11, S12, S13, S14, and S15. For LC-MS data in Figure 4 and supplementary Figure S10, see Data Availability section.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N.A.
Reporting on race, ethnicity, or other socially relevant groupings	N.A.
Population characteristics	N.A.
Recruitment	N.A.
Ethics oversight	N.A.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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

In this study, we hypothesize that light-dependent methionine synthesis and metabolism is tightly linked with growth by providing a potentially large flux of C1 transfers during biopolymer and metabolite synthesis and regulation via methylation. Moreover, synthesis of methylated pectin influences tissue morphogenesis and growth via subsequent demethylation on the primary cell wall, leading to changes in mechanical properties like elasticity and the associated release of methanol into the apoplast. We hypothesize that carbon for the activated methyl donor synthesis of AdoMet is directly produced by a 'photosynthetic C1 pathway' in chloroplasts initiated by the carboxylation reaction catalyzed by RuBisCO which generates 3-phosphoglyceric acid (PGA) then utilized by the plastidic phosphorylated serine pathway. Serine then transfers C1 units to THF which subsequently transfers C1 units to homocysteine during methionine synthesis. Followed by the export of methionine to the cytosol and its activation to AdoMet, the universal methyl group donor, a high flux of methyl transfer reaction might be sustained through an AdoMet recycling pathway in the cytosol involving the regeneration of homocysteine, independently of photorespiration. To test these hypotheses, we employed dynamic 13CO2-labelling of California poplar (Populus trichocarpa) leaves under controlled environmental conditions and whole branches under day/night light cycles together with analysis of stable carbon isotope ratios (13C/12C, δ13C values) of leaf phosphogyceric acid (PGA), serine, and methionine, cell wall methyl esters (OCH3), and leaf methanol (CH3OH), acetaldehyde (CH3CHO), and isoprene (C5H8) emissions (that is, C1, C2, C3 and C5 metabolism products). We compared 13CO2-branch labelling patterns under a 21% and 1% O2 atmosphere to evaluate the potential roles of C1-photosynthesis versus photorespiration in generating new cell wall methyl esters and the release of methanol during growth and development. We also evaluated P. tricocharpa leaf and branch gas exchange patterns in a field experiment to gain insight into potential temperature and hydraulic controls over diurnal growth and methanol emissions during the 2023 growing season in Berkeley, CA, USA. Finally, using comparative genomics, we examine the co-occurrence of proteins in the photosynthetic C1 pathway and trace the evolutionary origin of methionine synthase through cyanobacteria and algae to land plants.

Research sample

15 potted poplar trees were used for leaf (1-5 hour) and branch (2-5) day 13CO2 labelling under conditions which promoted high rates of net photosynthesis while suppressing net photosynthesis
.
1) Leaf 13CO2 labelling studies under constant environmental conditions for short time periods (up to 5 hours) promoting high rates of photosynthesis lasting 1 hr (n = 5), 2 hr (n = 2), 3 hr (n = 1), 4 hr (n = 2), and 5 hr (n = 2) were conducted together with online 13CO2 assimilation quantification and 13C/12C-methanol emission ratios analysis. In addition, short term (0, 5, 15, 30, 60, and 180 min) 13CO2 leaf labelling (replicated 2x for each time point) was conducted followed by flash freezing leaves at the end of the experiment for bulk cell wall pectin methyl ester 13C-analysis.

2) Branch 13CO2 labelling studies under diurnal light/dark cycles over 2 days (5 replicates) was performed with online 13CO2 assimilation quantification and 13C/12C-methanol emission ratios analysis and flash frozen at the end of the experiment for bulk cell wall pectin methyl ester and leaf metabolomics 13C-analysis were also performed. A single branch 13CO2 labelling study was conducted over 5 days and as well as a single study under 1% O2 over 2 days was also conducted.
A population of 20 field grown poplar trees was used to study the temperature and hydraulic effects of methanol emissions. One tree was randomly selected for 1-2 week branch gas exchange studies during April, June, and July 2023 as well as a single day experiments at the leaf level on one individual to quantify sap velocity and leaf gas exchange together with leaf water potential (from 10 trees) at midnight, predawn, mid-day, and early afternoon.

For the co-occcurance analysis of proteins of the photosynthetic C1 pathway and phylogenetic analysis of methionine synthase across the photosynthetic tree of life, all orthogroups of proteins of the listed genes in the C1 pathway were identified across all organisms available in STRING-DB.

To construct a phylogenetic tree, all protein sequences from the methionine synthase (MS) orthogroup were extracted from the STRING-DB and manually curated to filter sequences specific to two diatoms, two green algae, one moss and 15 land plants (dicots and monocots). Two cyanobacterial MS sequences were extracted from Cyanobase. In total, 76 sequences from 22 species were used to construct the tree.

Sampling strategy

No sample size calculations were performed. However, all replicate branch and leaf 13CO2 labelling experiments showed a strict-light-dependence of 13C-labelling of pectin methyl esters in primary cell walls, leaf methanol emissions, and targeted leaf metabolites.

1) Leaf 13CO2 labelling studies under constant environmental conditions for short time periods promoting high rates of photosynthesis lasting 1 hr (n = 5), 2 hr (n = 2), 3 hr (n = 1), 4 hr (n = 2), and 5 hr (n = 2) were conducted together with online 13CO2 assimilation quantification and 13C/12C-methanol emission ratios analysis and flash frozen at the end of the experiment for bulk cell wall pectin methyl ester 13C-analysis. All leaf samples showed a very tight linear correlations ($r^2 > 0.97$) between cumulative 13CO2 photoassimilation and the instantaneous 13C/12C-methanol emission ratio. The slope of the correlations were determined and shown to be similar between the different leaves, which derived from different branches on randomly selected trees.

2) Long-term (2 days) branch 13CO2 labeling over under diurnal light/dark cycles were repeated on randomly selected potted poplar trees 5 times, with each replicate showing similar light-dependent 13C-labelling patterns and magnitudes of methanol emissions as well as 13C-labelling of bulk leaf cell wall pectin methyl esters and targeted leaf metabolites extracted after the labeling period. All branch labelling experiments except one were successful. Leaves of one of the branches were damaged in the dark when installing the branch in the dynamic gas exchange chamber in the dark at midnight, so the experiment was aborted (data not collected). To demonstrate that the light-dependent 13C-labeling pattern of methanol emissions and cell pectin methyl esters could continue for a longer period of time, a single randomly selected potted tree was used to conduct branch 13CO2 labelling over 5 diurnal cycles. In addition, although all the branch 13CO2 labelling replicates under 21% O2 atmosphere are estimated to have low rates of photorespiration (due to moderate light and temperature, high soil moisture, and elevated 13CO2) such that Vo estimated to be <5% Vc, we conducted a single 2-day branch 13C-labelling experiment under 1% O2. In this single experiment which effectively eliminated

photorespiration (Vo estimated to be < 0.5% Vc), branch headspace was verified to be 1% and net O2 production was verified during the light period, and light-dependent 13C-labelling patterns and magnitudes of methanol emissions as well as 13C-labelling of bulk leaf cell wall pectin methyl esters was only slightly reduced relative to the branches under 21% O2. The slight decrease in the 13C-labeling of methanol emissions and pectin methyl esters was attributed to slight inhibition of photosynthesis under 1% O2 which included an activation of fermentation metabolism, demonstrated by greatly enhanced acetaldehyde emissions at night following darkening.
For the field experiments, 1-2 weeks of branch gas exchange studies on a single randomly selected tree as well as single day of diurnal leaf water potential and gas exchange studies were replicated three times throughout the growing season in April, June, and July of 2023. When all data were combined, a clear exponential dependence of methanol emissions and temperature was observed. All replicate studies were successful and no data were excluded from the field study.

Data collection

Kolby Jardine and to a lesser extent, postdoc Suman Som, were responsible for collecting and logging all gas exchange time series data for the branch and leaf 13CO2 labelling experiments as well as the field experiments. Luiza Gallo was responsible for manually downloading field temperature and sap velocity data as well as conducting all manual leaf water potential measurements using the pressure bomb technique. Trent R. Northen and Suzanne Kosina were responsible for all leaf metabolite extractions and LC-MS/MS analysis and Kolby Jardine purified whole leaf bulk cell wall material and shipped them to Germany where Frank Keppler and Markus Greule analyzed the pectin methyl esters. Finally, Melissa Roth and Shivani Upadhyaya conducted the cr.-occurance analysis of photosynthetic C1 pathway proteins as well as the phylogentic analysis of methioinine synthase across the photosynthetic tree of life. Kolby Jardine designed all graphic illustrations and worked with an animation specialist to develop the the C1 photosynthesis animation "Globe-to-Globe".

Timing and spatial scale

Leaf 13CO2 labelling studies under constant environmental conditions for short time periods (up to 5 hours) promoting high rates of photosynthesis lasting 1 hr (n = 5), 2 hr (n = 2), 3 hr (n = 1), 4 hr (n = 2), and 5 hr (n = 2) were conducted together with online 13CO2 assimilation quantification and 13C/12C-methanol emission ratios analysis. In addition, short term (0, 5, 15, 30, 60, and 180 min) 13CO2 leaf labelling (replicated 2x for each time point) was conducted followed by flash freezing leaves at the end of the experiment for bulk cell wall pectin methyl ester 13C-analysis.

Long-term (2 days) branch 13CO2 labeling over under diurnal light/dark cycles were repeated on randomly selected potted poplar trees 5 times, each time on a different randomly selected tree, following which leaf flash freezing allowed for leaf metabolite and pectin methyl ester 13C-analysis..

To demonstrate that the light-dependent 13C-labeling pattern of methanol emissions and cell pectin methyl esters could continue for a longer period of time, a single randomly selected potted tree was used to conduct branch 13CO2 labelling over 5 diurnal cycles followed by leaf flash freezing for pectin methyl ester 13C-analysis. In addition, we conducted a single branch 13C-labelling experiment over 2 diurnal cycles performed under 1% O2.

For the field experiments, 1-2 weeks of branch gas exchange studies as well as a single day diurnal leaf water potential and gas exchange studies were performed throughout the growing season in April, June, and July of 2023.

Data exclusions

No data were excluded from the analysis in this study.

Reproducibility

1) Leaf 13CO2 labelling studies under constant environmental conditions for short time periods (up to 5 hours) promoting high rates of photosynthesis lasting 1 hr (n = 5), 2 hr (n = 2), 3 hr (n = 1), 4 hr (n = 2), and 5 hr (n = 2) were conducted together with online 13CO2 assimilation quantification and 13C/12C-methanol emission ratios analysis. In addition, short term (0, 5, 15, 30, 60, and 180 min) 13CO2 leaf labelling (replicated 2x for each time point) was conducted followed by flash freezing leaves at the end of the experiment for bulk cell wall pectin methyl ester 13C-analysis. All experiments were successful and no data was excluded. All leaf studies showed a very tight linear correlation (r^2 > 0.97) between cumulative 13CO2 photoassimilation and the instantaneous 13C/12C-methanol emission ratio. The slope of the correlations were determined and shown to be very similar between the different leaves, which derived from different branches on randomly selected trees (out of 15). All experiments were successful and no data was excluded.
2) Long-term (2 days) branch 13CO2 labeling over under diurnal light/dark cycles were repeated on randomly selected potted poplar trees 5 times, with each replicate showing similar light-dependent 13C-labelling patterns and magnitudes of methanol emissions as well as 13C-labelling of bulk leaf cell wall pectin methyl esters and targeted leaf metabolites extracted after the labeling period. All branch labelling experiments except one were successful. Leaves of one of the branches were damaged in the dark when installing the branch in the dynamic gas exchange chamber at midnight and the experiment was aborted (data not collected). To demonstrate that the light-dependent 13C-labeling pattern of methanol emissions and cell pectin methyl esters could continue for a longer period of time, a single randomly selected potted tree was used to conduct branch 13CO2 labelling over 5 diurnal cycles. In addition, although all the branch 13CO2 labelling replicates under 21% O2 atmosphere are estimated to have low rates of photorespiration (due to moderate light and temperature, high soil moisture, and elevated 13CO2) such that Vo is estimated to be <5% of Vc, we conducted a single branch 13C-labelling experiment under 1% O2. In this single experiment which effectively eliminated photorespiration (Vo estimated to be < 0.5% Vc), net O2 production was verified in the light and light-dependent 13C-labelling patterns and magnitudes of methanol emissions as well as 13C-labelling of bulk leaf cell wall pectin methyl esters were only slightly reduced relative to the branches under 21% O2. The slight decrease in the 13C-labeling of methanol emissions and pectin methyl esters was attributed to inhibition of photosynthesis under 1% O2 which included an activation of fermentation metabolism observed here as highly elevated 13C2-acetaldheyde bursts following light-dark transitions which persisted all night.
3) For the field experiments, 1-2 weeks of branch gas exchange studies as well as single day diurnal leaf water potential and gas exchange studies were replicated three times throughout the growing season in April, June, and July of 2023. When all data were combined, a clear exponential dependence of methanol emissions and temperature was observed. All replicate studies were successful and no data were excluded from the field study.
4) For the co-occurance analysis of proteins of the photosynthetic C1 pathway and phylogenetic analysis of methionine synthase across the photosynthethic tree of life, all orthogroups of proteins of the listed genes in the C1 pathway were identified across all organisms available in STRING-DB.
5) To construct a phylogenetic tree, all protein sequences from the methionine synthase (MS) orthogroup were extracted from the STRING-DB and manually curated to filter sequences specific to two diatoms, two green algae, one moss and 15 land plants (dicots

and monocots). Two cyanobacterial MS sequences were extracted from Cyanobase. In total, 76 sequences from 22 species were used to construct the tree.

Randomization

The group of 15 potted poplar trees grown in the greenhouse and used for leaf and branch 13CO2 labeling studies were randomly selected for gas exchange, leaf metabolites, and cell wall studies. The only requirement was that the leaves selected for the leaf 13CO2 labeling study be dark green and maturing. During the branch gas exchange studies, a random potted tree was selected and moved into the analytical laboratory and placed under an LED grow light and automated watering system (and allowed to acclimate for serveral days). The branch selected for continuous 2-5 day gas exchange studies during 13CO2 labelling was also randomly selected, but was required to be in the upper canopy so that it was not shaded by branches above it. All branches studied had a mix of young light green immature leaves (near the end of the branch) and darker green maturing leaves.
For the field study, one tree was randomly selected out of 20 to conduct 1-2 weeks of continuous branch gas exchange and sap velocity (repeated 3 times during the growing season).

Blinding

Blinding was used when submitting frozen leaf samples for metabolite extraction and 13C-labelling analysis as well as bulk leaf cell wall 13C-analysis. All other datasets were collected by the corresponding author (K. Jardine) together with an undergraduate research assistant (Louiza Gallo) and postdoc (Suman Som), and blinding was not used.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions

Twenty California poplar saplings (Populus trichocarpa) were planted on May 24, 2021 in the Oxford Tract Experimental Facility field site. California poplar saplings were obtained from Plants of the Wild, WA, USA. Automated watering was configured during the growing season (April-December), such that 0.5 gal hr-1 was delivered to each tree for 1 hr each day at 8:00. However, during the warm summer months (June through October) watering duration increased to 1.5-3 hr in order to maintain relatively constant soil moisture. Prior to the commencement of experimentation in April 2023, the trees were grown in the field for 689 days (1 year, 10 months, and 20 days) yielding a height of 2.0-2.6 m.

Location

The poplar tree field experiment occurred at the Oxford Tract Experimental Facility field site at Latitude: 37.8733° N- Longitude: 122.2592° W- Elevation: approximately 130-150 meters (430-490 feet) above sea level.

Access & import/export

All greenhosue and field activities were conducted under the direct supervision of the Oxford Tract manger, Christina Wistrom, cwistrom@berkeley.edu who provided support and oversight on all tasks including safety and protocol training, receiving of plant material, establishment of plants in greenhouse space, supplemental lighting and environmental control, establishment of automated watering, pest control, and integrate with project researchers including the PI (Dr. Kolby Jardine), postdoctoral researcher, and poplar tree technician.

A detailed safety and ethics protocol for research was developed and approved both by the University of California Berkeley and Berkeley lab (BE-0161, Poplar esterified cell wall transformations and metabolic integration (PECTIN) study). At the end of the experiments, all plant material that was not utilized in experiments was autoclaved on site in-alkal disinfectant. In addition, all plant tissues/materials used in experiments were autoclaved and then disposed as compost which stayed on site. As poplar trees were grown for a maximum of a few years, the trees did not reach the maturity state of producing seeds and pollen. In addition, only wild-type poplar trees native of Washington state, USA were sued and no transgenic material was utilized. Frozen leaf samples were transported to the Joint BioEnergy Institute (JBEI) laboratory facilities on dry ice for extraction and analysis of leaf metabolites and purification of bulk leaf cell wall material. The dried and powdered cell wall extracts were stable at room temperature with low amounts from each leaf sample (e.g. 100 mg/leaf) shipped directly to Germany for analysis of cell wall methyl esters.

Disturbance

The Oxford Tract Experimental Facility field site is regularly utilized for growing plants for research and educational purposes at UC Berkeley. Following the end of the experiments in December 2023, all twenty trees were first dried following the cessation of watering, removed, with all biomass autoclaved prior to disposing through the UC system.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☐	☒ Plants

Methods

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

Dual use research of concern

Policy information about [dual use research of concern]

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
☒	☐ Public health
☒	☐ National security
☒	☐ Crops and/or livestock
☒	☐ Ecosystems
☒	☐ Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
☒	☐ Demonstrate how to render a vaccine ineffective
☒	☐ Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐ Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐ Increase transmissibility of a pathogen
☒	☐ Alter the host range of a pathogen
☒	☐ Enable evasion of diagnostic/detection modalities
☒	☐ Enable the weaponization of a biological agent or toxin
☒	☐ Any other potentially harmful combination of experiments and agents

Plants

Seed stocks

We used 15 potted wild-type California poplar (Populus trichocarpa) trees (average height of 2 meter) obtained as saplings (roughly 35 cm in height) from Plants of the Wild (Washington State, USA) and maintained for three years in the South Greenhouse at the Oxford Tract Experimental Facility in Berkeley, CA, USA, where they were regularly watered and subject to standard pest control practices. Water was delivered to each individual using an automated watering system in 15 gal pots containing Supersoil planting media (Scotts Co., Marysville, Ohio, USA). Ambient natural light was supplemented with LED lighting using an Argus Titan environmental control system with photocell (Argus Controls, British Columbia, Canada). The controller was programmed to turn LED lights off when detecting exterior light levels above 850 µmol m-2 s-1 during the 16-hr photoperiod (6:00 to 22:00).

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Only wild-type poplar trees were used in this study.