

Quinones as the Redox Signal for the Arc Two-Component System of Bacteria

Dimitris Georgellis, Ohsuk Kwon, Edmund C. C. Lin*

The Arc two-component signal transduction system mediates adaptive responses of *Escherichia coli* to changing respiratory conditions of growth. Under anaerobic conditions, the ArcB sensor kinase autophosphorylates and then transphosphorylates ArcA, a global transcriptional regulator that controls the expression of numerous operons involved in respiratory or fermentative metabolism. We show that oxidized forms of quinone electron carriers act as direct negative signals that inhibit autophosphorylation of ArcB during aerobic biosynthesis. Thus, the Arc signal transduction system provides a link between the electron transport chain and gene expression.

Two-component signal transduction systems are widespread in prokaryotes and play extensive roles in adaptation to environmental changes (1, 2). The Arc two-component system of *Escherichia coli*, comprising the ArcB transmembrane sensor kinase and the cytosolic ArcA response regulator, modulates the expression of numerous regulons and operons (the Arc modulon) in response to changes in redox conditions of growth (3–5). In contrast to typical sensor kinases that have a substantial periplasmic domain between two transmembrane segments on the NH₂-terminus for signal sensing, ArcB has a short periplasmic sequence of only 16 amino acids (6). ArcB is also unorthodox in having an elaborate cytosolic structure consisting of three catalytic domains: a primary transmitter with a conserved His residue (position 292), a receiver with a conserved Asp (position 576), and a secondary transmitter with a conserved His (position 717) (7, 8). ArcA is a classical response regulator that has an NH₂-terminal receiver domain with a conserved Asp residue (position 54) and a COOH-terminal helix-turn-helix domain for DNA binding.

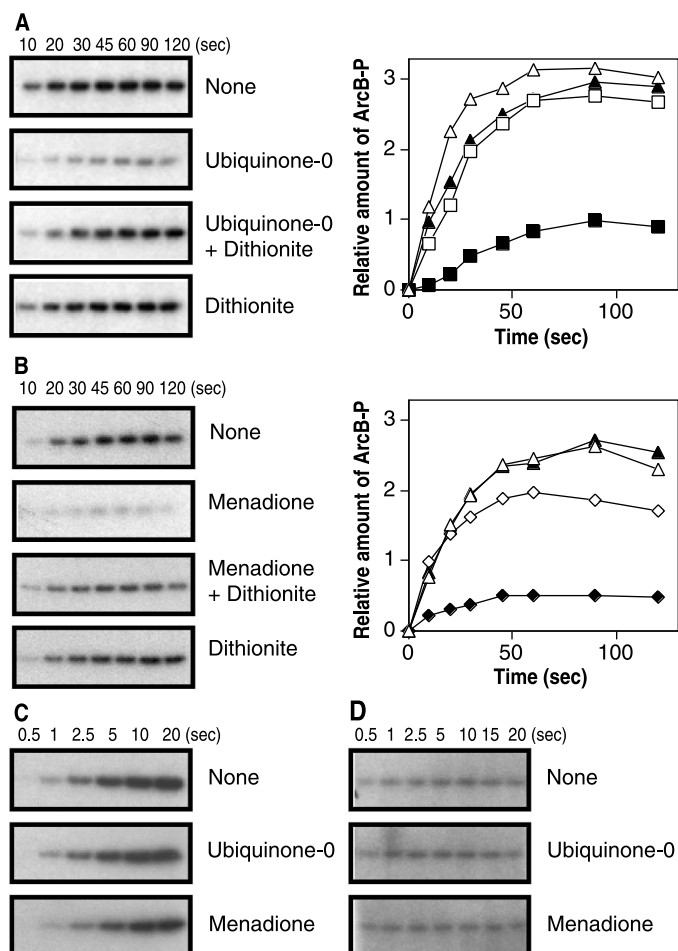
Under reducing conditions, ArcB autophosphorylates, then transphosphorylates ArcA through a His²⁹² → Asp⁵⁷⁶ → His⁷¹⁷ → Asp⁵⁴ phospho-relay, thereby increasing the affinity of ArcA for its DNA targets (9–11). Phosphorylated ArcA (ArcA-P) represses the expression of many genes involved in respiratory metabolism [e.g., enzymes of electron transport, the tricarboxylic acid cycle, and the glyoxalate shunt (3)] and activates other genes encoding proteins involved in fermentative metabolism [e.g., pyruvate formate lyase (12) and hydrogenase

(13)] by binding to a specific DNA sequence (14–16). Under oxidizing conditions, ArcB dephosphorylates ArcA-P through an Asp⁵⁴ → His⁷¹⁷ → Asp⁵⁷⁶ reverse phospho-relay (17).

Although much has been learned about the steps of signal transmission and decay as

well as the identity of numerous target operons, the actual signal for ArcB remains to be identified. Oxygen has been excluded as a direct signal for inhibiting autophosphorylation, because during anaerobic respiratory growth the ArcA-P-dependent repression of a target operon can be lifted by a supplemented electron acceptor in accordance with its oxidizing power (midpoint potential) (3). Several fermentation metabolites (e.g., D-lactate, pyruvate, and acetate) have been shown to enhance the autophosphorylation rate of ArcB in vitro by up to three times the basal level by acting at a site in the cytosolic domain (18, 19). However, these compounds are likely to be allosteric activators. The true signal is expected to curtail autophosphorylation under oxidizing conditions; otherwise, many target operons would remain under permanent repression. Moreover, the cellular levels of metabolites such as D-lactate depend not only on the respiratory state of the cell but also on the oxygen-hydrogen ratio of the carbohydrate being fermented. In addition, if the inputs come solely from the cytosol, the transmembrane domain of ArcB would not have evolved (or have been maintained). A

Fig. 1. Effects of ubiquinone-0 and menadione on the rate of ArcB autophosphorylation. (A) Purified ArcB⁷⁸⁻⁷⁷⁸ (2.5 μM) was incubated with [γ -³²P]ATP in the presence or absence of ubiquinone-0 (250 μM) and/or dithionite (500 μM). Left panel: Autoradiograms of the gels. Right panel: Net increase of ArcB-P with time in the absence (Δ) or presence of ubiquinone-0 (■), dithionite (▲), or ubiquinone-0 and dithionite (□). (B) Purified ArcB⁷⁸⁻⁷⁷⁸ was incubated with [γ -³²P]ATP in the presence or absence of menadione (1 mM) and/or dithionite (1 mM). Left panel: Autoradiograms of the gels. Right panel: Net increase of ArcB-P with time in the absence (Δ) or presence of menadione (◆), dithionite (▲), or menadione and dithionite (◇). (C) Purified CpxA¹⁸⁴⁻⁴⁵⁸ was incubated with [γ -³²P]ATP at room temperature in buffer A in the presence or absence of ubiquinone-0 (250 μM) or menadione (1 mM). (D) Purified BarA¹⁹⁸⁻⁹¹⁸ was incubated with [γ -³²P]ATP at room temperature in buffer A in the presence or absence of ubiquinone-0 (250 μM) or menadione (1 mM).



Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA.

*To whom correspondence should be addressed. E-mail: elin@hms.harvard.edu

case in point is the non-membrane-associated kinase NtrB that senses the state of nitrogen supply from cytosolic elements (20). We therefore explored the possibility that the transmembrane domain of ArcB either participates directly in signal reception or serves as an anchor to keep the protein close to the source of the signal. The former was excluded because the amino acid sequence of the transmembrane domain can be altered without impairing signal transduction (6). It thus appears that membrane association promotes interaction between the cytoplasmic portion of the sensor protein and a redox signal.

The major quinones—ubiquinone-8 (Q₈), menaquinone-8 (MK₈), and demethylmenaquinone (DMK₈)—are membrane-associated electron carriers that function as adapters between various electron-donating and electron-accepting enzyme complexes (21, 22) and were therefore assessed as possible direct signals for ArcB. When the soluble analogs ubiquinone-0 (Q₀) or menadione (MK₃) were incubated in the presence of [γ -³²P]adenosine triphosphate (ATP) and purified ArcB⁷⁸⁻⁷⁷⁸ [a truncated sensor kinase lacking the NH₂-terminal transmembrane domain (10)], autophosphorylation of the protein (23) was inhibited (Fig. 1, A and B). Half-maximal inhibition of ArcB⁷⁸⁻⁷⁷⁸ phosphorylation (24) by Q₀ occurred at about 5 μ M, whereas that by MK₃ occurred at about 50 μ M (25). Because quinones are reduced to quinols under fermentative conditions of growth, Q₀ and MK₃ were converted to their reduced form by treatment with excess dithionite (hydrosulfite) and then tested. Reduced Q₀, reduced MK₃, or dithionite alone did not affect the

rate of ArcB⁷⁸⁻⁷⁷⁸ autophosphorylation (Fig. 1, A and B). The quinones act at a site in either the linker or the first transmitter domain, because Q₀ also inhibited autophosphorylation of ArcB⁷⁸⁻⁵²⁰ (10), which lacks the receiver domain and the secondary transmitter domain (25). To test whether inhibition of the kinase activity by Q₀ and MK₃ is ArcB-specific, we examined two other sensor kinases: CpxA, a classical sensor kinase with a single transmitter domain (26), and BarA, which belongs to the ArcB subfamily of sensor kinases with three catalytic domains (27). Purified CpxA¹⁸⁴⁻⁴⁵⁸ (28) and BarA¹⁹⁸⁻⁹¹⁸ (29), both lacking the NH₂-terminal transmembrane domain, were used. The presence of Q₀ and MK₃ in the oxidized form did not alter the autophosphorylation rate of the two sensor kinases (Fig. 1, C and D).

The in vitro results suggest that the physiological redox state is signaled to ArcB by the oxidized form of quinone electron carriers. This would explain previous observations that in a mutant lacking both cytochrome bo₃ and cytochrome bd terminal oxidases, the aerobic expression of the ArcA-P-activatable Φ (*cyd-lacZ*) reporter was elevated, but that of the ArcA-P-repressible Φ (*cyo-lacZ*) reporter was lowered (30). In this mutant, the pool of Q₈ should be trapped in reduced form despite the oxidizing growth conditions. Because reduced quinones are unable to inhibit the kinase activity of ArcB, the level of ArcA-P should rise and accordingly alter the expression of the target operons.

To confirm the signaling role of Q₈ in vivo, we compared the aerobic expressions of Φ (*cydA'-lacZ*) in a *ubiCA* mutant that is blocked in the synthesis of the Q₈ precursor 3-octaprenyl-4-hydroxybenzoate, a *ubiCA arcB* double mutant, and the isogenic wild-type parent (31). Blockage of Q₈ synthesis increased the aerobic expression of Φ (*cydA'-lacZ*) by a factor of 3, but no increase was observed in the double mutant, indicating that increased aerobic expression of Φ (*cydA'-lacZ*) in the *ubiCA* mutant resulted from diminished inhibition of the ArcB kinase activity (Fig. 2). Together, the in vitro and in vivo data suggest that the oxidized forms of quinones are ArcB-specific signals that silence, rather than stimulate, ArcB kinase activity.

The quinones are ideally suited as redox signals for the Arc system, because the hydrophobicity provided by the isoprenoid chain not only ensures rapid lateral mobility within the membrane but may also facilitate limited vertical displacements, which could permit interactions of the reactive aromatic ring with the ArcB signal reception site. Signal reception by the cytosolic domain of a transmembrane protein is not unique for ArcB. The Aer transmembrane protein, the guiding element in bacterial aerotaxis, also lacks a periplasmic domain. This protein

senses the cellular redox state by a flavin adenine dinucleotide molecule bound to the cytosolic domain (32, 33).

Anchoring of the sensor domain close to the cytoplasmic membrane ensures that all of the ArcB molecules are within reach of the signal. Silencing most of the ArcB molecules would not be possible if they were randomly distributed in the cytosol. Indeed, we found that liberation of ArcB from the membrane results in a constitutively active kinase in vivo (6). A part of the structural evolution of ArcB can thus be viewed as a tinkering process that enables a sensor kinase to interact with an electron transport element as signal, without the intervention of a periplasmic domain. The attainment of this interaction also confers an additional role for the quinones to serve as signals for gene expression.

References and Notes

1. J. S. Parkinson, E. C. Kofoid, *Annu. Rev. Genet.* **26**, 71 (1992).
2. J. A. Hoch, T. J. Silhavy, *Two-Component Signal Transduction* (American Society for Microbiology, Washington, DC, 1995).
3. S. Iuchi, E. C. C. Lin, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1888 (1988).
4. A. S. Lynch, E. C. C. Lin, *Regulation of Gene Expression in Escherichia coli*, E. C. C. Lin, A. S. Lynch, Eds. (Landes, Georgetown, TX, 1996), p. 361.
5. ———, in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, F. C. Neidhardt et al., Eds. (American Society for Microbiology, Washington, DC, 1996), p. 1526.
6. O. Kwon, D. Georgellis, A. S. Lynch, D. Boyd, E. C. C. Lin, *J. Bacteriol.* **182**, 2960 (2000).
7. S. Iuchi, Z. Matsuda, T. Fujiwara, E. C. C. Lin, *Mol. Microbiol.* **4**, 715 (1990).
8. K. Ishige, S. Nagasawa, S.-I. Tokishita, T. Mizuno, *EMBO J.* **13**, 5195 (1994).
9. S. Iuchi, E. C. C. Lin, *J. Bacteriol.* **174**, 3972 (1992).
10. D. Georgellis, A. S. Lynch, E. C. C. Lin, *J. Bacteriol.* **179**, 5429 (1997).
11. O. Kwon, D. Georgellis, E. C. C. Lin, *J. Bacteriol.* **182**, 3858 (2000).
12. G. Sawers, B. Suppmann, *J. Bacteriol.* **174**, 3474 (1992).
13. L. Brondsted, T. Atlung, *J. Bacteriol.* **176**, 5423 (1994).
14. A. S. Lynch, E. C. C. Lin, *J. Bacteriol.* **178**, 6238 (1996).
15. M. T. Pellicer et al., *Mol. Gen. Genet.* **261**, 170 (1999).
16. A. M. McGuire, P. De Wulf, G. M. Church, E. C. C. Lin, *Mol. Microbiol.* **32**, 219 (1999).
17. D. Georgellis, O. Kwon, P. De Wulf, E. C. C. Lin, *J. Biol. Chem.* **273**, 32864 (1998).
18. S. Iuchi, *J. Biol. Chem.* **263**, 23972 (1993).
19. D. Georgellis, O. Kwon, E. C. C. Lin, *J. Biol. Chem.* **274**, 35950 (1999).
20. B. Magasanik, in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, F. C. Neidhardt et al., Eds. (American Society for Microbiology, Washington, DC, 1996), p. 1344.
21. R. B. Gennis, V. Stewart, in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, F. C. Neidhardt et al., Eds. (American Society for Microbiology, Washington, DC, 1996), p. 217.
22. Q₈ participates solely in aerobic respiration, whereas MK₈ and DMK₈ participate only in anaerobic respiration.
23. Unless otherwise specified, the phosphorylation assays were performed by incubating 2.5 μ M purified protein at 15°C with 40 μ M [γ -³²P]ATP (specific activity, 2 Ci/mmol) in buffer A [33 mM Hepes (pH 7.5), 50 mM KCl, 5 mM MgCl₂, 0.1 mM EDTA, and 10% glycerol] in the presence or absence of quinone and/or dithionite. When menadi-

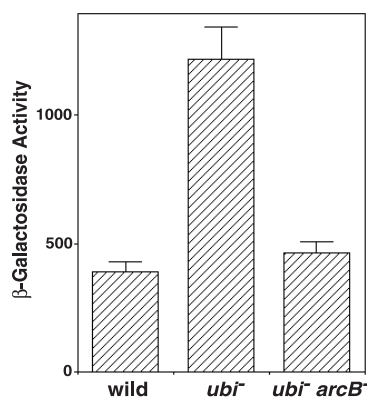


Fig. 2. Effects of ubiquinone-8 deficiency on the aerobic expression of Φ (*cydA'-lacZ*) operon fusion. Strains ECL5001 (*cyd*⁺ λ Φ (*cydA'-lacZ*)), ECL5039 (Δ *ubiCA*::Kan^r *cyd*⁺ λ Φ (*cydA'-lacZ*)), and ECL5040 (Δ *ubiCA*::Kan^r Δ *arcB*::Tet^r *cyd*⁺ λ Φ (*cydA'-lacZ*)) were grown in 5 ml of minimal medium (30) containing 40 mM D-galactose as sole carbon and energy source in 250-ml baffled flasks at 37°C with shaking (300 rpm). β -Galactosidase activity was assayed and is expressed in Miller units. The data are averages of four experiments; standard deviations are indicated.

one was tested, all reaction mixtures were supplemented with an appropriate amount of ethanol (5% v/v), because the menadione was dissolved in ethanol as a stock solution. The reaction was initiated by the addition of [γ - 32 P]ATP. Samples were withdrawn at the indicated time intervals, mixed with an equal volume of 2 \times SDS sample buffer, and immediately subjected to SDS-polyacrylamide gel electrophoresis on 12.5% polyacrylamide gels. The radioactivity of ArcB was revealed by exposure to X-Omat AR films, and the amount of protein-P was quantitated with a PhosphorImager.

24. Purified ArcB⁷⁸⁻⁷⁷⁸ (1 μ M) was incubated in the presence of increasing concentrations of ubiquinone-0 or menadione (1 μ M to 5 mM). The reaction was initiated by the addition of [γ - 32 P]ATP and terminated after 2 min.

25. D. Georgellis, O. Kwon, E. C. C. Lin, unpublished data.

26. P. M. Silverman, E. Wickersham, S. Rainwater, R. Harris, *J. Mol. Biol.* **220**, 271 (1991).

27. S. Nagasawa, S. Tokishita, H. Aiba, T. Mizuno, *Mol. Microbiol.* **6**, 799 (1992).

28. Plasmid pQE30CpxA¹⁸⁴⁻⁴⁵⁸, used for the expression of the His₆-tagged derivative of CpxA, was created as follows: Primers 5'-CCCGGATCCCATATGTGGA-GTCTGGCAAAAACCGGC-3' and 5'-GTGAAGCTTA-ACTCCGTTATACAGCGGC-3' were used in the polymerase chain reaction (PCR) with chromosomal DNA of strain MC4100 as the template. The PCR product was digested with Bam HI and Hind III and cloned between the Bam HI and Hind III sites of pQE30.

29. A.-K. Pernestig, Ö. Melefors, D. Georgellis, *J. Biol. Chem.* **276**, 225 (2001).

30. S. Iuchi, V. Chepuri, H.-A. Fu, R. B. Gennis, E. C. C. Lin, *J. Bacteriol.* **172**, 6020 (1990).

31. Strain ECL5039 (Δ ubiCA::Kan^r *cyd*⁺ λ Φ[*cydA'*-*lacZ*]) was constructed by P1 transduction of the Δ ubiCA::Kan^r allele from strain RKP4152 (34) to strain ECL5001 (*cyd*⁺ λ Φ[*cydA'*-*lacZ*]) (6). Strain

ECL5040 was constructed by P1 transduction of the Δ arcB::Tet^r allele from strain ECL5000 (6) to strain ECL5039.

32. S. I. Bibikov, R. Biran, K. E. Rudd, J. S. Parkinson, *J. Bacteriol.* **179**, 4075 (1997).

33. A. Rebbapragdala *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 10541 (1997).

34. B. Soballe, R. K. Poole, *Microbiology* **144**, 361 (1998).

35. We dedicate this work to Bernard D. Davis, who demonstrated the existence of the tricarboxylic acid cycle in bacteria and, in 1968, encouraged E.C.C.L. to study its genetic regulation. We thank R. Meganathan for discussions and R. Poole for strain RKP4152. Supported by U.S. Public Health Service grant GM40993 from the National Institute of General Medical Sciences.

29 January 2001; accepted 14 May 2001

Consistent Land- and Atmosphere-Based U.S. Carbon Sink Estimates

S. W. Pacala,^{1*} G. C. Hurtt,³ D. Baker,² P. Peylin,⁴
R. A. Houghton,⁵ R. A. Birdsey,⁶ L. Heath,⁶ E. T. Sundquist,⁷
R. F. Stallard,⁸ P. Ciais,⁹ P. Moorcroft,¹ J. P. Caspersen,¹
E. Shevliakova,¹ B. Moore,³ G. Kohlmaier,¹⁰ E. Holland,¹¹
M. Gloor,¹¹ M. E. Harmon,¹² S.-M. Fan,² J. L. Sarmiento,²
C. L. Goodale,¹³ D. Schimel,¹¹ C. B. Field¹³

For the period 1980–89, we estimate a carbon sink in the coterminous United States between 0.30 and 0.58 petagrams of carbon per year (petagrams of carbon = 10¹⁵ grams of carbon). The net carbon flux from the atmosphere to the land was higher, 0.37 to 0.71 petagrams of carbon per year, because a net flux of 0.07 to 0.13 petagrams of carbon per year was exported by rivers and commerce and returned to the atmosphere elsewhere. These land-based estimates are larger than those from previous studies (0.08 to 0.35 petagrams of carbon per year) because of the inclusion of additional processes and revised estimates of some component fluxes. Although component estimates are uncertain, about one-half of the total is outside the forest sector. We also estimated the sink using atmospheric models and the atmospheric concentration of carbon dioxide (the tracer-transport inversion method). The range of results from the atmosphere-based inversions contains the land-based estimates. Atmosphere- and land-based estimates are thus consistent, within the large ranges of uncertainty for both methods. Atmosphere-based results for 1980–89 are similar to those for 1985–89 and 1990–94, indicating a relatively stable U.S. sink throughout the period.

Despite widespread consensus about the existence of a terrestrial carbon sink of 1 to 2 Pg of C (Pg C) year⁻¹ in the Northern Hemisphere, the size, spatial distribution, and cause of the sink remain uncertain (1–3). Information about the sink comes from two primary sources: (i) atmosphere-based methods that determine the combination of carbon sources and sinks in an atmospheric transport model that gives the best match to a global set of atmospheric CO₂ data (the tracer-transport inversion method) and (ii) land-based approaches incorporating direct inventories of carbon on the ground, reconstructions of land

use change, and ecosystem models.

The size of the sink in temperate North America has been estimated with both approaches, with diverse results. One set of inverse modeling studies estimates a large North American sink of 1.7 Pg C year⁻¹ for 1988–92, with 1.4 Pg C year⁻¹ south of 51°N (4), whereas others estimate a much smaller sink (i.e., 0.5 Pg C year⁻¹ for the entire continent) (5–7). Land-based analyses for the United States in the 1980s suggest a sink of 0.08 to 0.35 Pg C year⁻¹, with virtually all of this in the coterminous United States (the United States minus Alaska and

Hawaii) (8–12). Although these land-based values are at least fourfold smaller than the Fan *et al.* estimate (4) for temperate North America, comparisons between existing land- and atmosphere-based estimates are not straightforward.

To make a direct comparison, atmospheric and land-based estimates should correspond to (i) the same time period, (ii) the same land area, and (iii) the same set of biogeochemical fluxes.

1) Inverse modeling studies show that global and North American sinks fluctuate among years by up to 100% of their long-term means (2, 3). This makes it essential to compare land- and atmosphere-based estimates from the same time period.

2) The portion of the atmosphere-based estimate in (4) attributable to the coterminous United States is only 48% of 1.7 Pg C year⁻¹ or 0.81 Pg C year⁻¹. This adjustment is based on the area of the coterminous United States as a fraction of North America (32%) and the assumption in (4) that the spatial distribution of the sink in any region matches the spatial

¹Department of Ecology and Evolutionary Biology, ²Atmospheric and Oceanic Sciences Program, Princeton University, Princeton, NJ 08544, USA. ³Complex Systems Research Center, Institute for the Study of Earth, Oceans and Space, University of New Hampshire, Durham, NH 03824, USA. ⁴Laboratoire de Biogéochimie Isotopique, UPMC-CNRS-INRA, Jussieu, Paris, France. ⁵Woods Hole Research Center, Post Office Box 296, Woods Hole, MA 02543, USA. ⁶U.S. Department of Agriculture Forest Service, Newtown Square, PA 19073, USA. ⁷U.S. Geological Survey, Quissett Campus, Woods Hole, MA 02543, USA. ⁸Water Resources Division, U.S. Geological Survey, Boulder, CO 80303-1066, USA. ⁹LSCE-CEA de Saclay, Orme des Merisiers, 91191 Gif/Yvette, France. ¹⁰Zentrum für Umweltforschung, Johann Wolfgang Goethe-Universität, 60325 Frankfurt, Germany. ¹¹Max-Planck-Institut für Biogeochemie, Carl Zeiss Promenade 10 07745 Jena, Germany. ¹²Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA. ¹³Department of Plant Biology, Carnegie Institution of Washington, Stanford, CA 94305-1297, USA.

*To whom correspondence should be addressed. E-mail: Pacala@princeton.edu