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Catchment properties and the photosynthetic trait composition of freshwater plant communities

Short title: Catchments rule aquatic plant traits

One sentence summary: The geographical distribution of bicarbonate use in freshwater plants is controlled by catchment characteristics.

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23 **ABSTRACT**

24 Unlike land plants, photosynthesis in many aquatic plants relies on bicarbonate in addition to

25 CO_2 to compensate for the low diffusivity and potential depletion of CO_2 in water.

26 Concentrations of bicarbonate and CO_2 vary greatly with catchment geology. Here we

27 investigate whether there is a link between these concentrations and the frequency of freshwater

28 plants possessing the bicarbonate use trait. We show, globally, that the frequency of plant species

29 with this trait increases with bicarbonate concentration. Regionally however, the frequency of

30 bicarbonate use is reduced at sites where the CO_2 concentration is substantially above air-

31 equilibrium consistent with this trait being an adaptation to carbon limitation. Future

32 anthropogenic changes of bicarbonate and CO_2 concentration may alter the species composition

33 of freshwater plant communities.

34

35 **MAIN TEXT**

36 The biogeography of terrestrial plants is influenced by climatic factors; primarily air temperature

37 and precipitation (*1*). Furthermore, the distribution of biochemical traits such as the two

38 terrestrial CO_2 concentrating mechanisms, C_4 photosynthesis and Crassulacean Acid

39 Metabolism, are linked to temperature and water availability (*2*). Although freshwater

40 angiosperms evolved from terrestrial ancestors (*3*), their growth is controlled by light, nutrients

41 and inorganic carbon (*4*) rather than water, and therefore the factors influencing their

42 biogeography is likely to be different. Inorganic carbon potentially limits photosynthesis in

43 aquatic systems, because the diffusion of CO_2 is 10^4-fold lower in water than in air.

44 Consequently, the CO_2 concentration needed to saturate photosynthesis is up to 12 times the air

45   equilibrium concentration (*5*). Moreover, rapid photosynthesis can reduce CO_2 in water

46   substantially below air saturation (*4*).

47

48   In response to carbon limitation, a few aquatic angiosperms evolved the same CO_2 concentrating

49   mechanisms found in their terrestrial ancestors, but the most frequent mechanism, found in about

50   half of studied submerged freshwater plants, is the exploitation of bicarbonate (HCO_3^-; (*4,6*)),

51   derived from mineral weathering of soils and rocks in the catchment. Bicarbonate is the

52   dominant form of inorganic carbon in fresh waters when pH is between ~6.3 and ~10.2, and its

53   concentration often exceeds that of CO_2 by 10- to 100-fold (*6*). The ability to use bicarbonate is

54   present in most taxonomic groups and appears to have evolved independently in cyanobacteria,

55   eukaryotic algae and vascular aquatic plants (*7*). This shows the fundamental importance of

56   bicarbonate use to plant fitness (*6*); increase of photosynthesis, growth and primary productivity

57   at higher bicarbonate concentrations has been documented (*8-10*). However, bicarbonate use is

58   not ubiquitous, because it involves costs as well as benefits. Costs include energy since it is an

59   active process (*11*) and rates of photosynthesis at limiting concentrations of inorganic carbon are

60   greater in CO_2 users than in bicarbonate users (*5,12*). Thus, where CO_2 concentrations are

61   substantially above air saturation, as is often the case in streams, the benefit of bicarbonate use

62   will be reduced (*13*). Furthermore, obligate CO_2 users can exploit alternative CO_2 sources in the

63   air, lake sediment or in the water overlying the sediment (*14*), allowing continued photosynthesis

64   without the need to invest in mechanisms required for bicarbonate use.

65

66   We hypothesized that since limitation of photosynthesis by inorganic carbon supply is

67   widespread in freshwater plants, the relative concentration of bicarbonate and CO_2 at a particular

68   site should determine the proportion of plants that are obligate CO_2 users *vs* bicarbonate users.

69   Since geochemical catchment characteristics determine bicarbonate concentration, there should

70   be broad biogeographical patterns in the proportion of freshwater plants able to use bicarbonate

71   while at a smaller scale, both the CO_2 and bicarbonate concentrations in lakes and streams might

72   structure the functional group composition.

73

74   To test these hypotheses, we generated a database of freshwater angiosperms and their ability to

75   use bicarbonate as an inorganic carbon source, based on data found in the literature. These were

76   complemented with new data we gathered on 35 species from mainly tropical regions where few

77   prior data existed (Table S1 and (*15*)). The resulting 131 species represent approximately 10%

78   of known species with a submerged life stage (*16*) and of these, 58 (44%) could use bicarbonate.

79   In order to quantify the distribution of bicarbonate users *vs* CO_2 users, we used: i) approximately

80   1 million geo-referenced plant records; ii) global plant ecoregion species lists; and iii) 963 site

81   specific plant compositions from northern hemisphere lakes and streams (Fig. S1). In each of the

82   investigated 963 sites, plant composition was related to measured concentration of CO_2 and

83   bicarbonate. The geo-referenced plant records and ecoregion species lists were linked to local

84   bicarbonate concentrations derived from a constructed global map of bicarbonate concentration

85   (Fig. S2 and (*15*)).

86

87   In the analyzed lake and stream sites, concentrations of both bicarbonate and CO_2 affected the

88   occurrence of obligate CO_2 users *vs* bicarbonate users, but differently within and between lakes

89   and streams (Fig. 1, and Fig. S3). The chance of observing a bicarbonate user in lakes and

90   streams correlated directly with concentrations of bicarbonate and CO_2 (ΔHabitat = -0.82 [-1.64;

4

91   0.01] (mean [95% confidence intervals]; Δ represents the difference between streams and lakes

92   in parameter estimates at the log(odds) scale, Fig S3)), Fig. 1A). However, with increasing

93   bicarbonate concentrations, the likelihood of observing a bicarbonate user increased in lakes, but

94   not in streams ($\Delta\beta_{Bicarbonate}$ = -0.82 [-1.10; -0.54] Fig. 1B; see (*15*) for an explanation of β).

95   Moreover, with an increase in CO_2, the chance of observing a bicarbonate user decreased in both

96   habitat types ($\Delta\beta_{CO2}$= -0.04 [-0.22; 0.13], Fig. 1C). The present study shows that the

97   concentration of bicarbonate has a different effect on the proportion of bicarbonate users in lakes

98   *vs* streams. Unlike in lakes, no relationship between bicarbonate availability and bicarbonate

99   users was found in streams. This upholds our hypothesis that where concentrations of CO_2 are

100   high, the competitive advantage of using bicarbonate as a carbon source for photosynthesis will

101   be reduced even if bicarbonate is available.

102

103   Across global plant regions (*17*), the shifting proportions of bicarbonate users *vs* obligate CO_2

104   users showed distinct spatial patterns (Fig. 2A). Compared to the overall mean, a higher

105   proportion of bicarbonate users was observed in Africa, temperate Asia, and the northern part of

106   North America (Fig. 2A). Globally, species utilizing bicarbonate were found in areas with higher

107   bicarbonate concentrations (bicarbonate users - CO_2 users = 0.16 [0.02; 0.30] mM; Fig. 2C; see

108   Fig. 3 for a local example). The proportion of bicarbonate using species increased with

109   bicarbonate concentrations within ecoregions (β = 0.14 [0.05; 0.24], (mean [95% confidence

110   limits]), Fig. 2B). Because catchment geology and geological history shape the distribution of

111   lakes and rivers, as well as the bicarbonate concentrations in freshwater ecosystems (*18,19*), they

112   are the chief determinants of plant distribution in freshwaters. CO_2 concentrations are largely

113   regulated by local CO_2 supersaturated inflow (*20*) and ecosystem metabolism, making modeling

114   difficult at large spatial scales (*19,21*). Thus, future models of freshwater CO_2 concentrations

115   may improve the prediction of plant distributions even further. Although global lake and river

116   data exist to some extent as annual means (*22*), given the temporal variability in CO_2

117   concentration, the appropriate concentration would be that during the growing season at the

118   specific site (*20*).

119

120   Anthropogenic changes as a consequence of deforestation, cultivation of land, application of

121   nitrate fertilizers and reduced atmospheric acid deposition (*23*) are causing large scale increases

122   in bicarbonate concentrations (*24,25*). The observed increasing bicarbonate concentrations are

123   expected to cause a severe impact on bicarbonate poor lakes, because higher bicarbonate

124   concentrations will markedly change species composition (*26*) by allowing tall, fast growing

125   bicarbonate users to colonize and suppress smaller species adapted to the use of CO_2 alone in or

126   near the sediment (*27*). There is evidence for re-establishment of species that are able to use

127   bicarbonate, after bicarbonate has increased because of liming (*28*) or as a result of reduction in

128   acid deposition (*29*). Moreover, systematic changes in species composition caused by changes in

129   CO_2 concentration has also been demonstrated in a river system where the proportion of CO_2

130   users declined as CO_2 decreased downstream (*13*). In contrast, increasing atmospheric CO_2

131   concentrations, even if they influence dissolved CO_2, will have little effect on the abundance of

132   bicarbonate users, since increases in CO_2 will be small relative to bicarbonate concentrations and

133   will have little effect on plant photosynthesis rate (*30*).

134

135   Our study shows that bicarbonate use by aquatic angiosperms is widespread in fresh waters

136   around the globe, and that the proportion of obligate CO_2 users to bicarbonate users is

137   significantly related to the bicarbonate concentration. Among terrestrial plants, the evolution of

138   leaf traits and different photosynthetic pathways that enable rapid carbon assimilation and

139   improved water economy (*31*) has resulted in global biogeographical patterns that are linked to

140   variations in climate (*32,33*). In contrast, for freshwater plants, we show that biogeographical

141   patterns of bicarbonate use exist and that these are caused by catchment properties that determine

142   the concentration of bicarbonate and CO_2. This insight will help evaluate the repercussions of

143   future changes in concentration of bicarbonate and CO_2 on the biodiversity and ecosystem

144   function for fresh waters.

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146   **REFERENCES AND NOTES**
147
148   1.   M. C. Peel, B. L. Finlayson, T. A. McMahon, Updated world map of the Köppen-Geiger
149   climate classification. *Hydro. Earth Syst. Sci. Discus.* **4**, 439-473 (2007).
150   2.   C. J. Still, J. A. Berry, G. J. Collatz, R. S. DeFries, Global distribution of C_3 and C_4
151   vegetation: carbon cycle implications. *Global Biogeochem. Cycles* **17**, 6-1 (2003).
152   3.   D. H. Les, N. P. Tippery, In time and with water ... the systematics of alismatid
153   monocotyledons. *Early Events Monocot Evol.* **83**, 118-164 (2013).
154   4.   T. V. Madsen, S. C. Maberly, Diurnal variation in light and carbon limitation of
155   photosynthesis by two species of submerged freshwater macrophyte with a differential
156   ability to use bicarbonate. *Freshw. Biol.* **26**, 175-187 (1991).
157   5.   S. C. Maberly, T. V. Madsen, Affinity for CO_2 in relation to the ability of freshwater
158   macrophytes to use HCO_3^-. *Func. Ecol.* **12**, 99-106 (1998).
159   6.   S. C. Maberly, B. Gontero, Ecological imperatives for aquatic CO_2-concentrating
160   mechanisms. *J. Exp. Bot.* **68**, 3797-3814 (2017).
161   7.   M. Giordano, J. Beardall, J. A. Raven, CO_2 concentrating mechanisms in algae:
162   mechanisms, environmental modulation, and evolution. *Annu. Rev. Plant Biol.* **56**, 99-
163   131 (2005).
164   8.   K. Sand-Jensen, H. Frost-Christensen, Photosynthesis of amphibious and obligately
165   submerged plants in CO_2-rich lowland streams. *Oecologia* **117**, 31-39 (1998).
166   9.   M. R. Andersen, T. Kragh, K. Sand-Jensen, Extreme diel oxygen and carbon cycles in
167   shallow vegetated lakes. *Proc. R. Soc. B Biol. Sci.* **284**, 20171427 (2017).
168   10. T. V. Madsen, K. Sand-Jensen, Photosynthetic capacity, bicarbonate affinity and growth
169   of *Elodea canadensis* exposed to different concentrations of inorganic carbon. *Oikos* **50**,
170   176-182 (1987).
171   11. J. A. Raven, J. Beardall, M. Giordano, Energy costs of carbon dioxide concentrating
172   mechanisms in aquatic organisms. *Photosynth. Res.* **121**, 111-124 (2014).

173   12.   T. V. Madsen, S. C. Maberly, High internal resistance to CO_2 uptake by submerged
174   macrophytes that use HCO_3^-: measurements in air, nitrogen and helium. *Photosynth.*
175   *Res.* **77**, 183-190 (2003).
176   13.   S. C. Maberly, S. A. Berthelot, A. W. Stott, B. Gontero, Adaptation by macrophytes to
177   inorganic carbon down a river with naturally variable concentrations of CO_2. *J. Plant.*
178   *Physiol.* **172**, 120-127 (2015).
179   14.   T. V. Madsen, K. Sand-Jensen, Photosynthetic carbon assimilation in aquatic
180   macrophytes. *Aquat. Bot.* **41**, 5-40 (1991).
181   15.   See supplementary materials.
182   16.   K. Murphy *et al.*, World distribution, diversity and endemism of aquatic macrophytes.
183   *Aquat. Bot.* **158**, 103127 (2019).
184   17.   R. K. Brummitt, F. Pando, S. Hollis, N. A. Brummitt, *World geographical scheme for*
185   *recording plant distributions*. (International Working Group on Taxonomic Databases for
186   Plant Sciences (TDWG …, 2001).
187   18.   R. Lauerwald, J. Hartmann, N. Moosdorf, S. Kempe, P. A. Raymond, What controls the
188   spatial patterns of the riverine carbonate system?—A case study for North America.
189   *Chem. Geol.* **337**, 114-127 (2013).
190   19.   R. Marcé *et al.*, Carbonate weathering as a driver of CO_2 supersaturation in lakes. *Nat.*
191   *Geosci.* **8**, 107-111 (2015).
192   20.   S. C. Maberly, P. A. Barker, A. W. Stott, M. M. De Ville, Catchment productivity controls
193   CO_2 emissions from lakes. *Nat. Clim. Change* **3**, 391 (2013).
194   21.   L. J. Tranvik *et al.*, Lakes and reservoirs as regulators of carbon cycling and climate.
195   *Limnol. Oceanogr.* **54**, 2298-2314 (2009).
196   22.   P. A. Raymond *et al.*, Global carbon dioxide emissions from inland waters. *Nature* **503**,
197   355 (2013).
198   23.   P. A. Raymond, S. K. Hamilton, Anthropogenic influences on riverine fluxes of dissolved
199   inorganic carbon to the oceans. *Limnol. Oceanogr. Let.* **3**, 143-155 (2018).
200   24.   P. A. Raymond, N.-H. Oh, R. E. Turner, W. Broussard, Anthropogenically enhanced fluxes
201   of water and carbon from the Mississippi River. *Nature* **451**, 449 (2008).
202   25.   J. L. Stoddard *et al.*, Regional trends in aquatic recovery from acidification in North
203   America and Europe. *Nature* **401**, 575 (1999).
204   26.   O. Vestergaard, K. Sand-Jensen, Alkalinity and trophic state regulate aquatic plant
205   distribution in Danish lakes. *Aquat. Bot.* **67**, 85-107 (2000).
206   27.   K. Sand-Jensen, M. Sondergaard, Phytoplankton and epiphyte development and their
207   shading effect on submerged macrophytes in lakes of different nutrient status.
208   *Internationale Revue Der Gesamten Hydrobiologie* **66**, 529-552 (1981).
209   28.   T. E. Brandrud, Effects of liming on aquatic macrophytes, with emphasis on Scandinavia.
210   *Aquat. Bot.* **73**, 395-404 (2002).
211   29.   D. T. Monteith *et al.*, Biological responses to the chemical recovery of acidified fresh
212   waters in the UK. *Environ. Pollut.* **137**, 83-101 (2005).
213   30.   G. Bowes, Facing the inevitable: plants and increasing atmospheric CO_2. *Annu. Rev. Plant*
214   *Biol.* **44**, 309-332 (1993).
215   31.   I. J. Wright *et al.*, The worldwide leaf economics spectrum. *Nature* **428**, 821-827 (2004).

216 32. H. Lambers, F. S. Chapin Iii, T. L. Pons, *Plant physiological ecology*. (Springer Science &
217 Business Media, 2008).
218 33. I. J. Wright *et al.*, Global climatic drivers of leaf size. *Science* **357**, 917-921 (2017).
219 34. R. Smart, J. Barko, Laboratory culture of submersed freshwater macrophytes on natural
220 sediments. *Aquat. Bot.* **21**, 251-263 (1985).
221 35. S. C. Maberly, D. H. N. Spence, Photosynthetic inorganic carbon use by freshwater
222 plants. *J. Ecol.* **71**, 705-724 (1983).
223 36. T. Hengl *et al.*, SoilGrids250m: Global gridded soil information based on machine
224 learning. *PLoS one* **12**, e0169748 (2017).
225 37. R. M. Newton, J. Weintraub, R. April, The relationship between surface water chemistry
226 and geology in the North Branch of the Moose River. *Biogeochem.* **3**, 21-35 (1987).
227 38. A. Zeileis, F. Cribari-Neto, B. Grün, I. Kos-midis, Beta regression in R. *J. Stat. Soft.* **34**, 1-24
228 (2010).
229 39. M. Van Kleunen *et al.*, Global exchange and accumulation of non-native plants. *Nature*
230 **525**, 100 (2015).
231 40. J. Alahuhta *et al.*, Global variation in the beta diversity of lake macrophytes is driven by
232 environmental heterogeneity rather than latitude. *J. Biogeogr.* **44**, 1758-1769 (2017).
233 41. L. Baastrup-Spohr, K. Sand-Jensen, S. C. H. Olesen, H. H. Bruun, Recovery of lake
234 vegetation following reduced eutrophication and acidification. *Freshw. Biol.* **62**, 1847-
235 1857 (2017).
236 42. S. J. Moe, A. Schmidt-Kloiber, B. J. Dudley, D. Hering, The WISER way of organising
237 ecological data from European rivers, lakes, transitional and coastal waters. *Hydrobiol.*
238 **704**, 11-28 (2013).
239 43. T. Riis, K. Sand-Jensen, O. Vestergaard, Plant communities in lowland Danish streams:
240 species composition and environmental factors. *Aquat. Bot.* **66**, 255-272 (2000).
241 44. M. T. Furse *et al.*, *The ecological status of European rivers: evaluation and
242 intercalibration of assessment methods*. (Springer Science & Business Media, 2009), vol.
243 188.
244 45. S. Birk, N. Willby, Towards harmonization of ecological quality classification: establishing
245 common grounds in European macrophyte assessment for rivers. *Hydrobiol.* **652**, 149-
246 163 (2010).
247 46. E. Lewis, D. Wallace, L. J. Allison, "Program developed for CO_2 system calculations,"
248 (Brookhaven National Lab., Dept. of Applied Science, Upton, NY, 1998).
249 47. D. N. Karger *et al.*, Climatologies at high resolution for the earth's land surface areas. *Sci.*
250 *Data* **4**, 170122 (2017).
251 48. J. E. Titus, A. M. Pagano, Carbon dioxide and submersed macrophytes in lakes: linking
252 functional ecology to community composition. *Ecology* **98**, 3096-3105 (2017).
253 49. S. N. Wood, *Generalized additive models: an introduction with R*. (Chapman and
254 Hall/CRC, 2017).
255 50. J. Heino, J. Alahuhta, S. Fattorini, Phylogenetic diversity of regional beetle faunas at high
256 latitudes: patterns, drivers and chance along ecological gradients. *Biodivers. Conserv.* **24**,
257 2751-2767 (2015).
258 51. O. Schweiger, S. Klotz, W. Durka, I. Kühn, A comparative test of phylogenetic diversity
259 indices. *Oecologia* **157**, 485-495 (2008).

260 52. K. R. Clarke, R. M. Warwick, A taxonomic distinctness index and its statistical properties.
261 *J. Appl. Ecol.* **35**, 523-531 (1998).
262 53. L. Adamec, Mineral nutrition of carnivorous plants: A review. *Bot. Rev.* **63**, 273-299
263 (1997).
264 54. K. Sand-Jensen, M. F. Pedersen, S. L. Nielsen, Photosynthetic use of inorganic carbon
265 among primary and secondary water plants in streams. *Freshw. Biol.* **27**, 283-293 (1992).
266 55. L. Yin, W. Li, T. V. Madsen, S. C. Maberly, G. Bowes, Photosynthetic inorganic carbon
267 acquisition in 30 freshwater macrophytes. *Aquat. Bot.* **140**, 48-54 (2017).
268 56. E. L. Smith, The influence of light and carbon dioxide on photosynthesis. *J. Gen. Phys.* **20**,
269 807-830 (1937).
270 57. S. C. Maberly, T. V. Madsen, Freshwater angiosperm carbon concentrating mechanisms:
271 processes and patterns. *Funct. Plant Biol.* **29**, 393-405 (2002).
272 58. J. E. Keeley, Photosynthetic pathway diversity in a seasonal pool community. *Func. Ecol.*
273 **13**, 106-118 (1999).
274 59. J. R. Newman, J. A. Raven, Photosynthetic carbon assimilation by *Crassula helmsii*.
275 *Oecologia* **101**, 494-499 (1995).
276 60. S. A. Pierini, S. M. Thomaz, Effects of inorganic carbon source on photosynthetic rates of
277 *Egeria najas* Planchon and *Egeria densa* Planchon (Hydrocharitaceae). *Aquat. Bot.* **78**,
278 135-146 (2004).
279 61. J. T. Bain, M. C. F. Proctor, The requirement of aquatic bryophytes for free CO_2 as an
280 inorganic carbon source: some experimental evidence. *New Phytol.* **86**, 393-400 (1980).
281 62. D. R. Webb, M. R. Rattray, J. M. A. Brown, A preliminary survey for crassulacean acid
282 metabolism (CAM) in submerged aquatic macrophytes in New Zealand. *N. Z. J. Mar.*
283 *Freshw. Res.* **22**, 231-235 (1988).
284 63. T. V. Madsen, S. C. Maberly, G. Bowes, Photosynthetic acclimation of submersed
285 angiosperms to CO_2 and HCO_3^-. *Aquat. Bot.* **53**, 15-30 (1996).
286 64. J. I. Jones, J. Eaton, K. Hardwick, Physiological plasticity in *Elodea nuttallii* (Planch.) St.
287 John. *J. Aquat. Plant Man.* **31**, 88-88 (1993).
288 65. H. L. Boston, M. S. Adams, T. P. Pienkowski, Utilization of sediment CO_2 by selected
289 North American isoetids. *Ann. Bot.* **60**, 485-494 (1987).
290 66. A. M. Farmer, D. H. N. Spence, Studies of diurnal acid fluctuations in British isoetid-type
291 submerged aquatic macrophytes. *Ann. Bot.* **56**, 347-350 (1985).
292 67. J. A. Raven *et al.*, The role of CO_2 uptake by roots and CAM in acquiaition of inorganic C
293 by plants of the isoetid life-form - a review, with new data on *Eriocaulon decangulare* L
294 *New Phytol.* **108**, 125-148 (1988).
295 68. T. K. Van, W. T. Haller, G. Bowes, Comparison of the photosynthetic characteristics of
296 three submersed aquatic plants. *Plant Physiol.* **58**, 761-768 (1976).
297 69. M. E. Salvucci, G. Bowes, Induction of reduced photorespiratory activity in submersed
298 and amphibious aquatic macrophytes. *Plant Physiol.* **67**, 335-340 (1981).
299 70. W. Spencer, G. Bowes, *Limnophila* and *Hygrophila*: a review and physiological
300 assessment of their weed potential in Florida. *J. Aquat. Plant Man.* **23**, 7-16 (1985).
301 71. D. H. N. Spence, S. C. Maberly, in *Carbon uptake by aquatic photosynthetic organisms,*
302 W. J. Lucas, J. L. Berry, Eds. (American Society of Plant Physiologists, Rockville, 1985), pp.
303 125-143.

304 72. S. Wium-Andersen, Photosynthetic uptake of free CO_2, by the roots of *Lobelia*
305   *dortmanna*. *Physiol. Plant.* **25**, 245-248 (1971).
306 73. H. B. A. Prins, J. F. H. Snel, R. J. Helder, P. E. Zanstra, Photosynthetic HCO_3^- utilization
307   and OH^- excretion in aquatic angiosperms: light-induced pH changes at the leaf surface.
308   *Plant Physiol.* **66**, 818-822 (1980).
309 74. B. Hyldgaard, H. Brix, Plasticity in carbon acquisition of the heterophyllous *Luronium*
310   *natans*: An endangered freshwater species in Europe. *Aquat. Bot.* **94**, 127-133 (2011).
311 75. P. T. Orr, J. Pokorný, P. Denny, P. J. M. Sale, Photosynthetic response of *Myriophyllum*
312   *salsugineum* A.E. Orchard to photon irradiance, temperature and external free CO_2.
313   *Aquat. Bot.* **30**, 363-378 (1988).
314 76. J. E. Keeley, D. R. Sandquist, Carbon: freshwater plants. *Plant Cell Environ.* **15**, 1021-1035
315   (1992).
316 77. Y. Zhang *et al.*, Biochemical and biophysical CO_2 concentrating mechanisms in two
317   species of freshwater macrophyte within the genus *Ottelia* (Hydrocharitaceae).
318   *Photosynth. Res.* **121**, 285-297 (2014).
319 78. Y. Kadono, Photosynthetic carbon sources in some *Potamogeton* species. *Bot. Mag.* **93**,
320   185-194 (1980).
321 79. A. J. M. Smits, M. J. H. De Lyon, G. Van Der Velde, P. L. M. Steentjes, J. G. M. Roelofs,
322   Distribution of three nymphaeid macrophytes (*Nymphaea alba* L., *Nuphar lutea* (L.) Sm.
323   and *Nymphoides peltata* (Gmel.) O. Kuntze) in relation to alkalinity and uptake of
324   inorganic carbon. *Aquat. Bot.* **32**, 45-62 (1988).
325 80. M. Bodner, Inorganic carbon source for photosynthesis in the aquatic macrophytes
326   *Potamogeton natans* and *Ranunculus fluitans*. *Aquat. Bot.* **48**, 109-120 (1994).
327 81. E. D. Allen, D. H. N. Spence, The differential ability of aquatic plants to utilize the
328   inorganic carbon supply in fresh waters. *New Phytol.* **87**, 269-283 (1981).
329 82. P. Denny, D. C. Weeks, Effects of light and bicarbonate on membrane potential in
330   *Potamogeton schweinfurthii* (Benn.). *Ann. Bot.* **34**, 483-496 (1970).
331 83. J. M. Bristow, The effects of carbon dioxide on the growth and development of
332   amphibious plants. *Can. J. Bot.* **47**, 1803-1807 (1969).
333 84. J. A. Raven, A. M. Johnston, J. R. Newman, C. M. Scrimgeour, Inorganic carbon
334   acquisition by aquatic photolithoatrophs of the Dighty Burn, Angus, U.K.: uses and
335   limitations of natural abundance measurements of carbon isotopes. *New Phytol.* **127**,
336   271-286 (1994).
337 85. S. Beer, R. G. Wetzel, Photosynthetic carbon metabolism in the submerged aquatic
338   angiosperm *Scirpus subterminalis*. *Plant Sci. Let.* **21**, 199-207 (1981).
339 86. H. B. A. Prins, M. B. de Guia, Carbon source of the water soldier, *Stratiotes aloides* L.
340   *Aquat. Bot.* **26**, 225-234 (1986).
341 87. L. Adamec, Photosynthetic inorganic carbon use by aquatic carnivorous plants. *Carn.*
342   *Plant Newsl.*, 50-53 (1995).
343 88. R. E. Moeller, Carbon-uptake by the submerged hydrophyte *Utricularia purpurea*. *Aquat.*
344   *Bot.* **5**, 209-216 (1978).
345 89. J. E. Titus, W. H. Stone, Photosynthetic response of two submersed macrophytes to
346   dissolved inorganic carbon concentration and pH. *Limnol. Oceanogr.* **27**, 151-160 (1982).

90.   S. Loczy, R. Carignan, D. Planas, The role of roots in carbon uptake by the submersed
        macrophytes *Myriophyllum spicatum*, *Vallisneria americana*, and *Heteranthera dubia*.
        Hydrobiol. **98**, 3-7 (1983).

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framed the research questions, and wrote the manuscript, with input from the working group

(A.B.H., J.A., A.B-P., P.B., P.A.C., F.E., T.F., J.H., T.S.J, S.J.M., T.R., L.S. and O.V.). L.L.I.

analyzed the data and prepared the figures. A.B.H and O.P. performed the pH-drift experiments

and together with A.W. searched the literature for bicarbonate uptake in aquatic plants. A.W.,

L.L.I., and L.B-S. assembled the data for the global analysis. F.E., L.B-S, L.S., S.C.M., S.J.M,

J.A., and T.F. assembled the site-specific lake data and the site-specific stream data was

assembled by A. B.-P., P.B., P.A.C., D.G., K.S.-J., T.R, T.S.J., and O.V., A.W., L.L.I., and L. B-

S. prepared the site-specific data for further analysis.

372

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374

Data availability: All R scripts and cleaned datasets used for this analysis are available at the
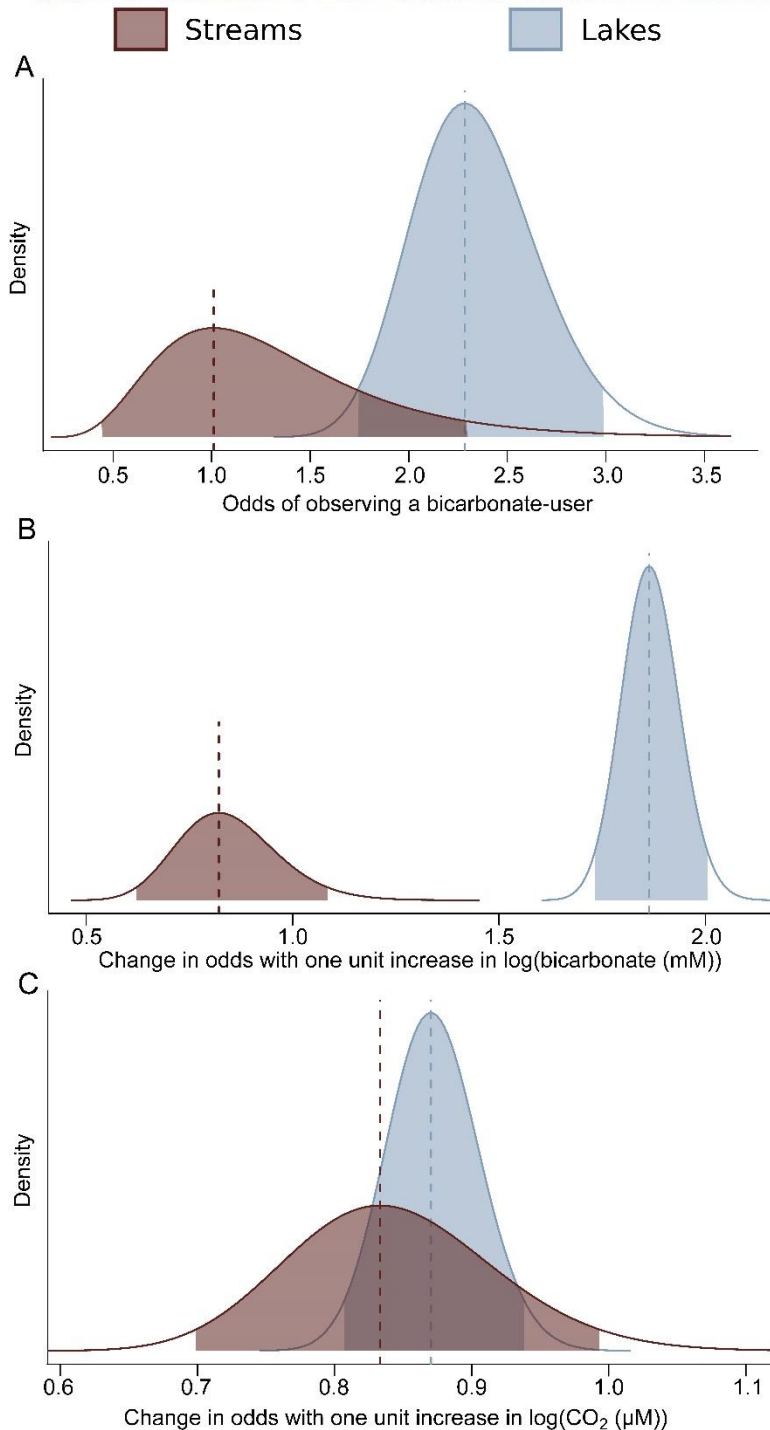
Dryad Digital Repository.

Fig. 1

Bicarbonate use in submerged freshwater plant communities. (**A**) likelihood of observing a bicarbonate user *vs* a CO_2 user in streams (n=172, red) and lakes (n=791, blue); (**B** and **C**), modeled odds of observing a bicarbonate user *vs* a CO_2 user as a function of bicarbonate (**B**) and CO_2 (**C**) concentrations. Values > 1 indicate a higher likelihood (**A**) or increase in likelihood (**B** and **C**) of observing a bicarbonate user *vs* a CO_2 user with a one unit increase in bicarbonate (**B**) and CO_2 concentrations (**C**). The dotted vertical lines show mean estimates and shaded areas the 95% confidence limits around the mean.

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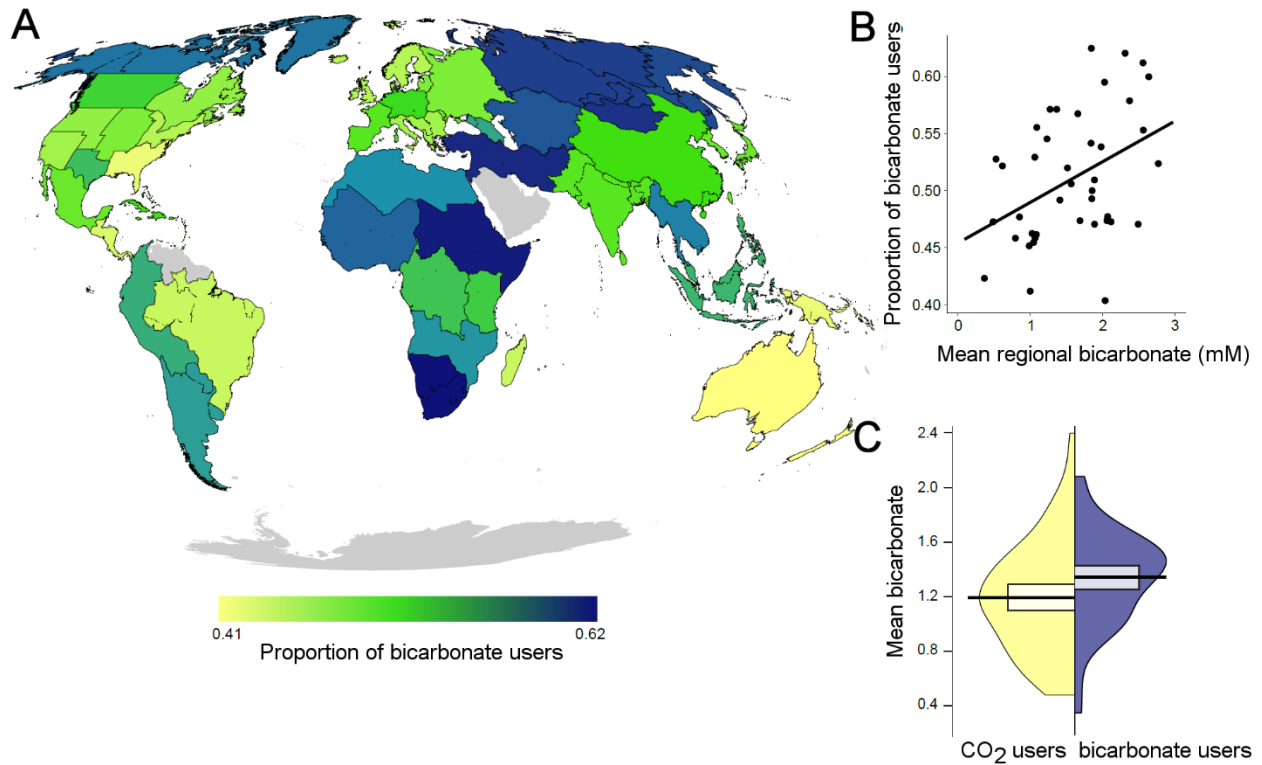
414
415
416 **Fig. 2**

417 **Global relationship between bicarbonate and the proportion of bicarbonate users in**

418 **freshwater plants.** (**A**) Proportion of bicarbonate using species across 52 plant ecoregions. Grey

419 areas indicate regions where information on bicarbonate use in local plants is not available. (**B**)

420 Relationship between mean bicarbonate concentration in plant regions and frequency of

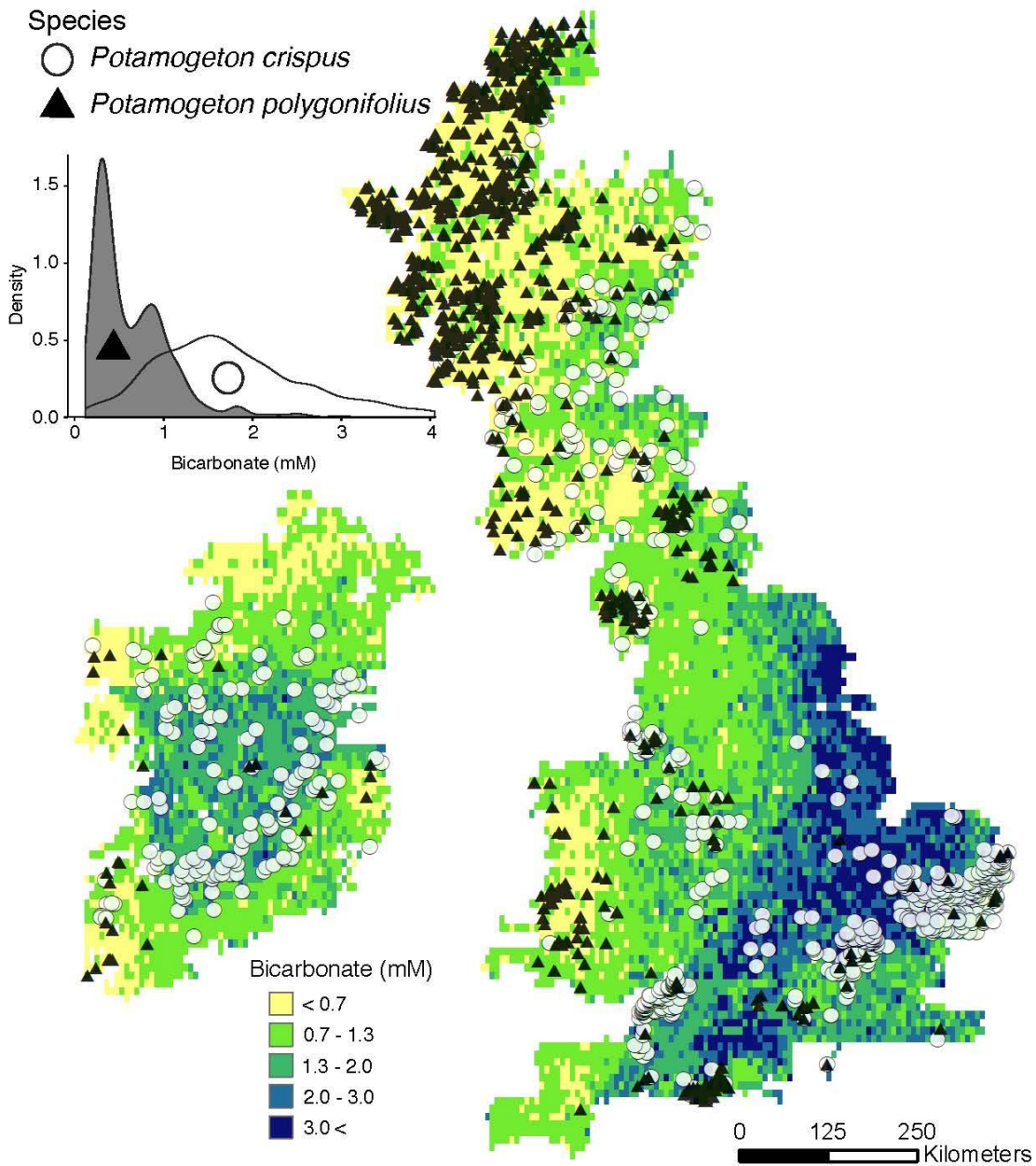421 bicarbonate users. The line represents the mean proportion of bicarbonate users. (**C**) Density

422 plots of bicarbonate preferences for bicarbonate users ($n = 57$) and obligate CO_2 users ($n = 72$).

423 The central horizontal black line represents the mean and the boxes indicate the 95% confidence

424 intervals around the mean.

425
426

427
428
429 **Fig. 3**

430 **Steep gradients in bicarbonate concentrations and spatial separation in species distribution**

431 **in the British Isles**. Distribution of two pondweed species with contrasting bicarbonate use in

432 the British Isles. *Potamogeton polygonifolius* (obligate CO_2 user, black triangles) is found in

433 areas with lower bicarbonate concentrations compared to *Potamogeton crispus* (bicarbonate user,

434 white circles). The top left insert shows the density distribution of the two species across

435   bicarbonate concentrations. Bicarbonate concentrations are from the global bicarbonate map

436   (Fig. S2) and species data were extracted from the geo-referenced plant occurrences (*15*).

437

438   **Supplementary Materials**

439   Materials and Methods (*15*).

440   References (*34-90*).

441   Fig. S1 - Site-specific observations of bicarbonate use.

442   Fig. S2 - Global bicarbonate map.

443   Fig. S3 - The probability of observing bicarbonate use in a species at 963 study sites.

444   Fig. S4 - Overview of *in situ* lake bicarbonate measurements.

445   Fig. S5 - Variable importance plot of the Random Forest modelling global bicarbonate

446   concentrations.

447   Fig. S6 - Partial dependence plots of the eight variables used to model global bicarbonate

448   concentrations.

449   Fig. S7 - Histogram of taxonomic distinctness for 1000 random subsamples of a fixed number of

450   131 species drawn from a common species pool.

451   Table S1 - List of freshwater angiosperms and their trait of inorganic carbon use.

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Science

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Supplementary Materials for

Catchment properties and the photosynthetic trait composition of freshwater plant communities

P Iversen, L. L., Winkel, A., Baastrup-Spohr, L., Hinke, A. B., Alahuhta, J., Baattrup-Pedersen, A., Birk, S., Brodersen, P., Chambers, P A., Ecke, F., Feldmann, T., Gebler, D., Heino, J., Jespersen, T S., Moe, S J., Riis, T, Sass, L., Vestergaard, O., Maberly, S C., Sand-Jensen, K., Pedersen, O.

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This PDF file includes:

> Materials and Methods
> Figs. S1 to S7
> Table S1

Other Supplementary Materials for this manuscript include the following:

> Data S1 All R scripts and cleaned datasets used for this analysis are available at the Dryad Digital Repository.

Materials and Methods

Traits of inorganic carbon use in aquatic plants

Information on species ability to use bicarbonate was collected from the literature and *ex-situ* pH drift experiments. A comprehensive scientific literature search was conducted in order to identify and collect data on inorganic carbon use of aquatic angiosperms, resulting in the identification of 104 species with validated information. Nomenclature follows a *sensu* plant list: www.theplantlist.org.

Tropical plant species for experiments were received from an aquatic plant nursery (Tropica Aquarium plants, Egå, Denmark). To ensure expression of bicarbonate use in species capable of doing so, plants were acclimated for 14 days in water with a high bicarbonate concentration (2 mM HCO_3) under air equilibrium of CO_2 (~20 µM CO_2) in a temperature controlled room (20 °C). To ensure temperature stability, each aquarium was equipped with a heating element and temperature was increased to 23 °C (± 1). Temperature was logged using a HOBO temperature/light sensor (HOBO Pendant, Onset Computer, Bourne, USA). The light regime was 12 h-light/12 h-darkness, with an average photon irradiance of 350 µmol photons m^{-2} s^{-1} (PAR) provided by fluorescent tubes (Luminux cool daylight 39 W/865 HO, Osram GmbH, Munich, Germany). Nine individual plants of each species were divided into three 30 L aquaria (three plants in each). The medium was a modified Smart & Barko (*34*) solution with 75% $KHCO_3$ and 25% $NaHCO_3$ to ensure adequate potassium concentration. The medium was changed twice a week. When possible, plants were kept in the pots with stone wool in which they had been grown by the producer. When this was not possible, dependent on the plant morphology, plants were either planted in sand (0.6-1 mm grain size) or weighed down using a ceramic ring. pH drift experiments were used to identify the ability of plant species to use bicarbonate as a source of inorganic carbon (*35*). The pH drift experiment relies on the fact that any carbon taken up by a plant results in an increase in pH without influencing alkalinity. If pH increases to more than 9.4, the species is classified as a bicarbonate user as almost no CO_2 is available (< 1 µM at 20 °C and with an alkalinity of 1 meq. L^{-1}) and it is inferred that HCO_3^- is being used as a source of inorganic carbon.

Leaf segments weighing 60 mg (± 5 mg) fresh mass were taken from the youngest fully developed leaf and cleared of any present epiphytes. The incubation medium was the same as that to which the plants had been acclimated (modified Smart & Barko solution, 2 mM HCO_3^-). Oxygen concentration was reduced to 20% of air equilibrium by bubbling with N_2 to avoid photorespiration due to high O_2 concentration from photosynthesis. Twenty-five ml glass vials, containing two glass beads to ensure stirring, were used to incubate leaf tissue. Vials with leaves were mounted on a vertically rotating wheel in a temperature controlled water bath at 25 °C for 17 hours with an average photon irradiance of 555 µmol photons m^{-2} s^{-1} (Phillips Master, TL-D, 18W/840, Phillips, AE Eindhoven, Holland) measured with a 4π sensor (US-SQS/L, Walz GmbH, Effeltrich, Germany). After incubation, pH was measured with a pH electrode (403-M8_s7/120. Rfill 9811, Mettler Toledo, Glostrup, Denmark; PHM 92, Radiometer, Brønshøj, Denmark).

A global bicarbonate map

The global distribution of bicarbonate was estimated from existing global maps of runoff accumulated freshwater alkalinity (*19*) and soil pH in water (*36*) (Fig. S2). This analytical

approach was chosen in order to match site-specific plant observations to local bicarbonate concentrations. Given the heterogeneous distribution of specific habitats for each of our study species, we expected that local bicarbonate concentration would be the product of downstream routing of alkalinity through the watershed, and that local deviations from these accumulations would have been caused by local soil properties (*37*). Based on one layer of accumulated alkalinity (*19*) and seven layers of soil pH in water (cross section of standard depths 0, 5, 15, 30, 60, 100 and 200 cm; (*36*), we modelled 1806 site-specific measures of bicarbonate (gathered from a global dataset; see Fig. S4). Given that bicarbonate typically accounts for >95% of alkalinity in water (*6*), we used site-specific alkalinity as a proxy for bicarbonate concentration. The 1806 *in situ* bicarbonate concentrations were derived from two data sources: The GEMSTAT database (www.gemstat.org) and the WISE4 database of the European Environmental Agency (https://www.eea.europa.eu/data-and-maps/data/waterbase-water-quality). Following a previous study (*6*), we removed unrealistically high alkalinity values (> 5 meq. L^{-1}) and calculated mean alkalinity per site. The global map of runoff accumulated freshwater alkalinity (*19*) slightly underestimates high values of alkalinity, because it did not account for concentrating mechanisms such as evaporation. To prevent violating the premises of input data for the new bicarbonate map (Fig. S2), we excluded alkalinity values of more than 5 meq. L^{-1} from the site-specific data prior to analyses. Finally, we calculated mean values per site for the subsequent analyses.

We used random forest models to model site-specific bicarbonate concentrations. Random forest is a nonparametric, machine learning regression tree combining individual decision trees (in this case, 500 trees) into a single ensemble model capable of fitting complex relationships with high predictive performance (*38*). Our random forest model was built using empirical bicarbonate measures as the response variable (to be modelled) and the corresponding values of the eight global layers as predicting variables. Each random forest model was run with a fixed set of 500 trees and a flexible number of predictors tried at each node (value set to the number of predictors divided by three). Consistency in error rates was visually inspected by plotting error rates against the number of trees in the ensemble. Across all models, the error rate stabilized between 50 and 100 trees. Model performance was evaluated by randomly splitting the 1806 measures into a training set (75%) and a test set (25%). The training data were used to build the model and the test data as an "out-of-bag" sample to compare observed and predicted bicarbonate values. From 1000 repetitions, a mean Pearson product moment correlation (ppmc) coefficient between observed and predicted bicarbonate was calculated for random forest models built on the runoff accumulated alkalinity layer, the seven pH soil maps, and a combination of all layers. The random forest model built on all eight layers produced the highest concordance between observed and predicted bicarbonate values (mean ppmc 0.73, Fig. S2B) supporting the initial inclusion of both accumulated runoff alkalinity and soil pH in the model. Alkalinity and lower levels of soil pH had the highest contribution to the model (Fig. S5) increasing with increasing bicarbonate concentrations (Fig. S6). Using the global coverage of the alkalinity and 7 soil pH maps, and the random forest model, global measures of bicarbonate were predicted at a 1/16 degree spatial resolution reflecting the highest common resolution of the input data (Fig. S2A).

The global occurrence of bicarbonate users

Following recent global plant trait censuses (*39*), we used the world geographical scheme for recording plant distributions (TDWG; *1*) to map the occurrence of all 131 study species. For each

studied species, we compiled occurrence data for the 52 subcontinental regions of TDWG extracted from the World Checklist of Selected Plant Families (http://apps.kew.org/wcsp/), supplemented with data from the Germplasm Resources Information Network (https://www.ars-grin.gov). We updated these occurrences with geo-referenced records from the Global Biodiversity Information Facility (GBIF; http://gbif.org [downloaded 3rd November 2016]). From a raw set of GBIF records, we removed non-geo-referenced and overlapping records within species, as well as obvious outliers (e.g. records from botanical gardens), creating a final dataset of 1,017,608 geo-referenced records. From these we calculated average bicarbonate values for each species based on a spatial overlay between plant records and the bicarbonate map (Fig. S2A). We evaluated differences in average bicarbonate preferences between bicarbonate users and obligate CO_2 users in a Gaussian linear contrast model.

The spatial extent of bicarbonate users *vs* obligate CO_2 users was analyzed by calculating the proportion of bicarbonate users in 52 TDWG regions. When present, the relationship between the proportion of bicarbonate users and mean bicarbonate concentration (estimated from the global bicarbonate map) in climate regions was analyzed via a beta regression model (*38*) by a logit link, with bicarbonate concentration as a linear predictor and the frequency of species using bicarbonate as the response variable. Statistical significance was evaluated by inspecting the 95% confidence estimates of the slope parameter in the regression model.

From a collective dataset of 963 sites (Fig. S1), we used site-specific observations of our 131 studied species to explore how the presence of bicarbonate user species were affected by local variations in CO_2 and bicarbonate availability. The site-specific data originates from regional and national surveys of aquatic plants and water chemistry (pH and alkalinity) in lakes (*40-42*) and streams (*43-45*) that were also monitored for water chemistry variables. Site-specific CO_2 concentrations were calculated based on alkalinity, pH and temperature according to (*46*). Site-specific temperatures were extracted from a high resolution (30 arc sec) land surface climate model (*47*) as mean temperature in the warmest quarter.

While increasing bicarbonate has been shown to increase the proportion of bicarbonate users in lakes (*26*), a similarly strong positive pattern has not been found in streams (*43*). In contrast, studies in both streams and lakes have shown that increasing CO_2 concentration positively affects the presence of obligate CO_2 users (and thus potentially decreasing the proportion of bicarbonate users) independently of bicarbonate concentration (*13,48*). From this, we predicted that on a large spatial scale, the probability of observing a bicarbonate user increases logarithmically with an increase in bicarbonate concentration and decreases logarithmically with an increase in CO_2 (*5*). We evaluated our prior expectations in a multivariate model using the observed presence of bicarbonate and obligate CO_2 users across the 963 sites (having removed two sites with alkalinity measurements below zero).

A multivariable logistic regression model was created, containing interactions between i) habitat type (stream or lake) and bicarbonate concentration; and ii) habitat type and CO_2 concentration as explanatory variables. These parameter interactions were used to test, on a log-odds scale, for differences in the effects of bicarbonate and CO_2 concentrations on the probability of observing a species with bicarbonate use (do the observed species utilize bicarbonate or not). Initial model runs indicated the presence of spatial autocorrelation. Thus, we accounted for residual patterns of

spatial autocorrelation by adding a two dimensional spherical spline based on geographic coordinates in the model (*49*). Based on Moran's I autocorrelation coefficient of the residual outputs, the final model did not show any indications of spatial autocorrelation (observed Moran's I = -0.0026, Null expectation = -0.0001, P-value = 0.12). Statistical significance in the final model was evaluated by inspecting the 95% confidence estimates of the slope parameter in the regression model. Any significant interaction parameters would suggest different response to CO_2 or bicarbonate gradients between lakes and streams (*16*). Parameter estimates for lakes and streams (as shown in Fig. 1) were derived from the full model estimates (Fig. S3) and shown as odds and change in odds (Fig .1).

The 131 species with information about their bicarbonate use represent about 10% of the 1297 species known to have a predominantly fully submerged life stage (*16*), species list provided by Kevin Murphy. Upscaling the results of this study would require the 131 species to consist of a random sample from the phylogenetic tree of plants. We tested this assumption by comparing our sample from the global list of 1297 species. Owing to lack of a true phylogeny of all aquatic plants of the world, we used taxonomic hierarchies as a proxy for phylogenetic relationships; this approach has been used in several studies dealing with phylogenetic diversity (e.g., *50,51*). We used Taxonomic Distinctness (TD), measuring the taxonomic distances between species in an assemblage, as a proxy for phylogenetic diversity (*52*). We used equal branch lengths and four taxonomic levels for the calculation of taxonomic distances between species: genus, family, order, and class for all of the 1297 species. Following Clarke & Warwick (*52*), we compared the observed TD with the values expected by randomly sampling 131 species from the entire species pool of 1297. Accordance between our observed TD and the random estimates indicates that the species included in our study represent a random taxonomic sample of the entire species pool. The observed TD of our 131 study species (82.7) did not differ from what would be expected by randomly selecting 131 species from the species pool (95% quantiles from 1000 simulations = 82.0-86.5, Fig. S7).

Fig. S1.
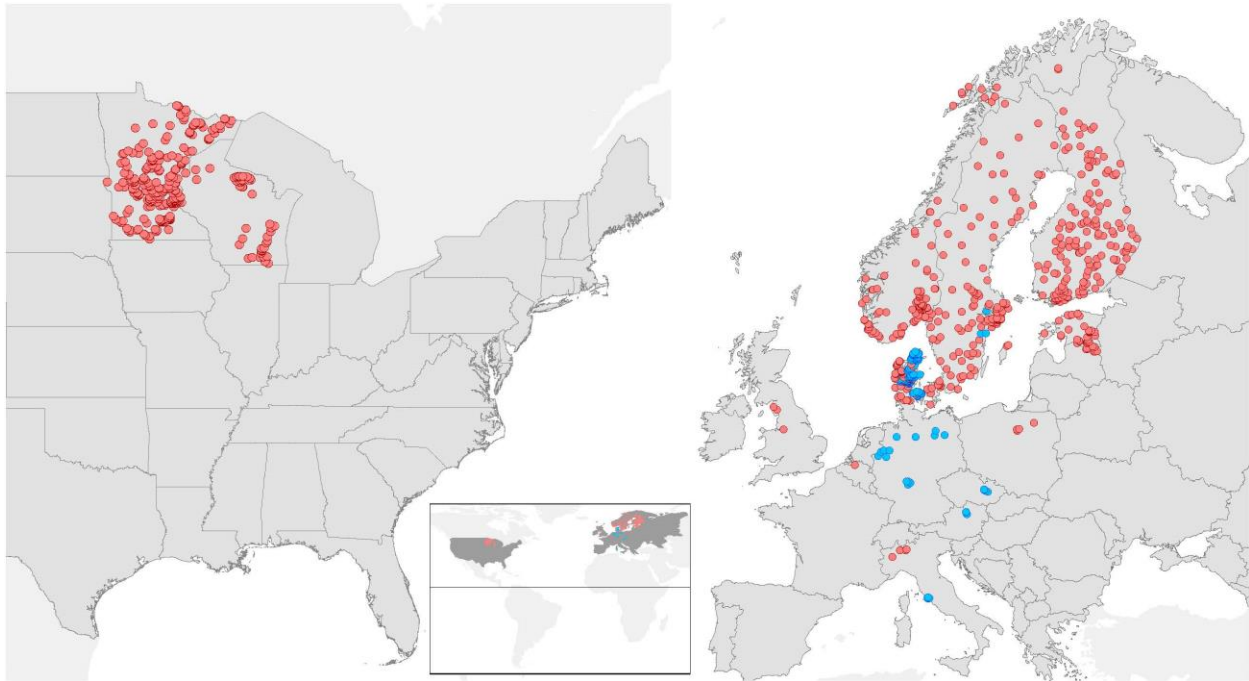
Site-specific observations of bicarbonate use. Spatial distribution of 963 sites used to explore how the presence of species able to use bicarbonate was affected by local variations in CO_2 and bicarbonate concentration. Red dots represent lakes (n=791) and blue dots represent streams (n=172).

Fig. S2.
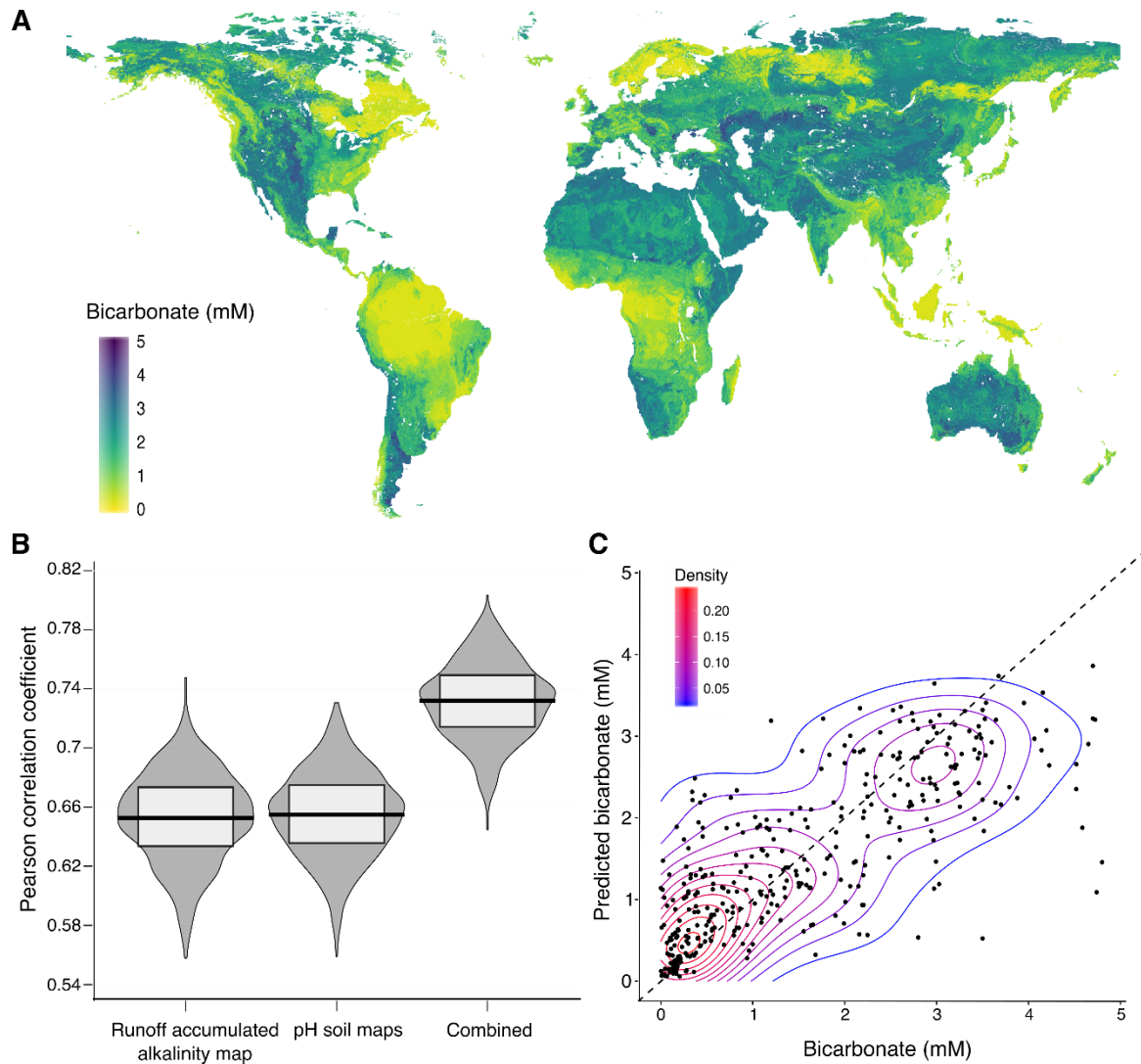
Global bicarbonate map. (**A**) Global bicarbonate map with 300×300 meter resolution modeled from bicarbonate data in 1806 lakes (obtained from GEMSTAT database (www.gemstat.org)) and 7 layers of soil pH using a random forest model. (**B**) Pearson product moment correlation coefficient of observed *vs* predicted bicarbonate using 3 different models. (**C**) Concentration of predicted bicarbonate as a function of observed bicarbonate.

Fig. S3.
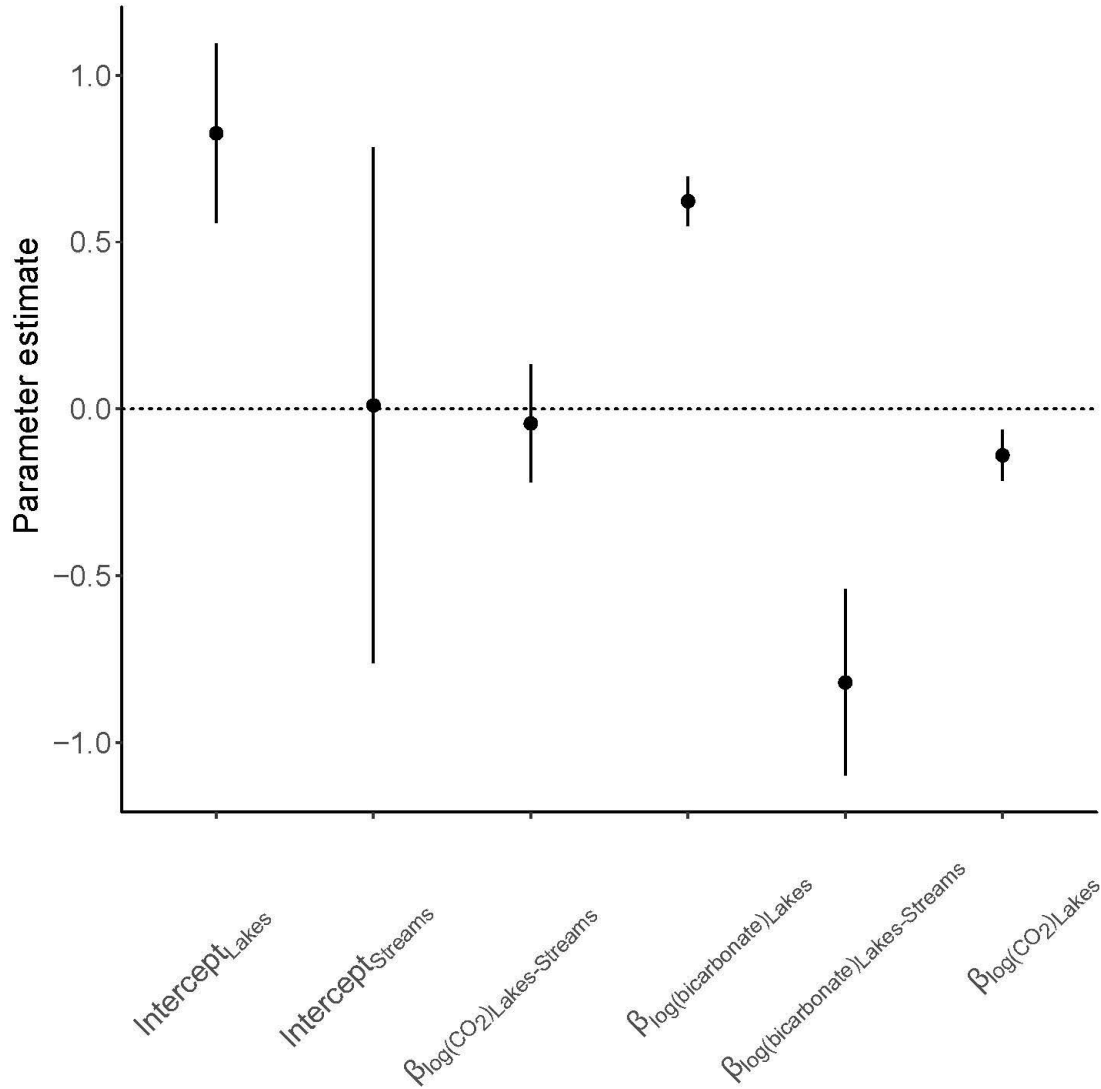
**The probability of observing bicarbonate use in a species at 963 study sites.** Parameter estimates from a multiple logistic regression model, including habitat type (stream or lake), log(bicarbonate), and log(CO_2) as explanatory variables. The dots represent means of log (odds) (for the intercepts) and change in log(odds) (for the slope parameters). Lines depict the 95% confidence limits of the mean.

Fig. S4.
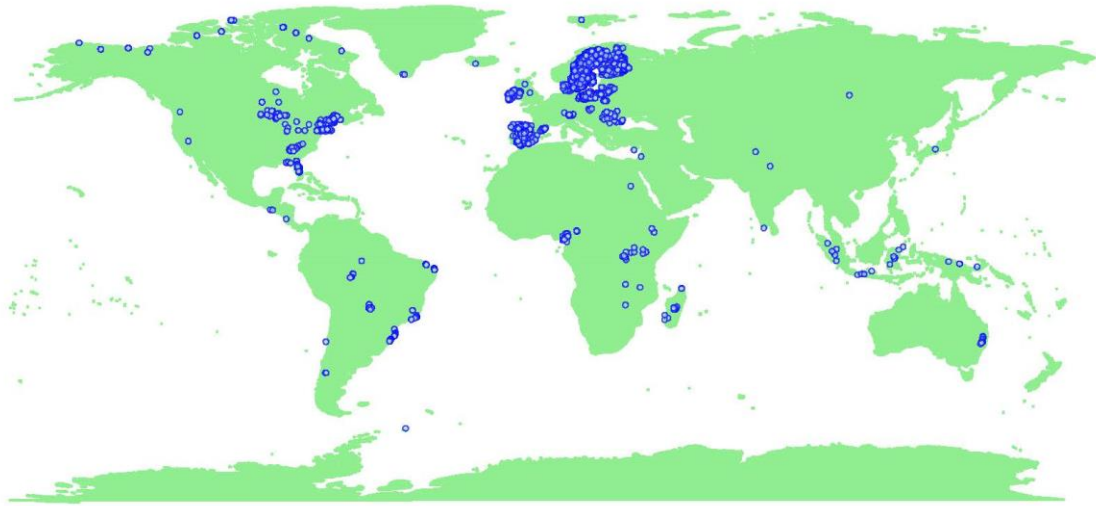
Overview of *in situ* lake bicarbonate measurements. Data taken from the GEMSTAT database (www.gemstat.org) and the European Environmental Agency (https://www.eea.europa.eu) provided bicarbonate data from six continents.

Fig. S5.
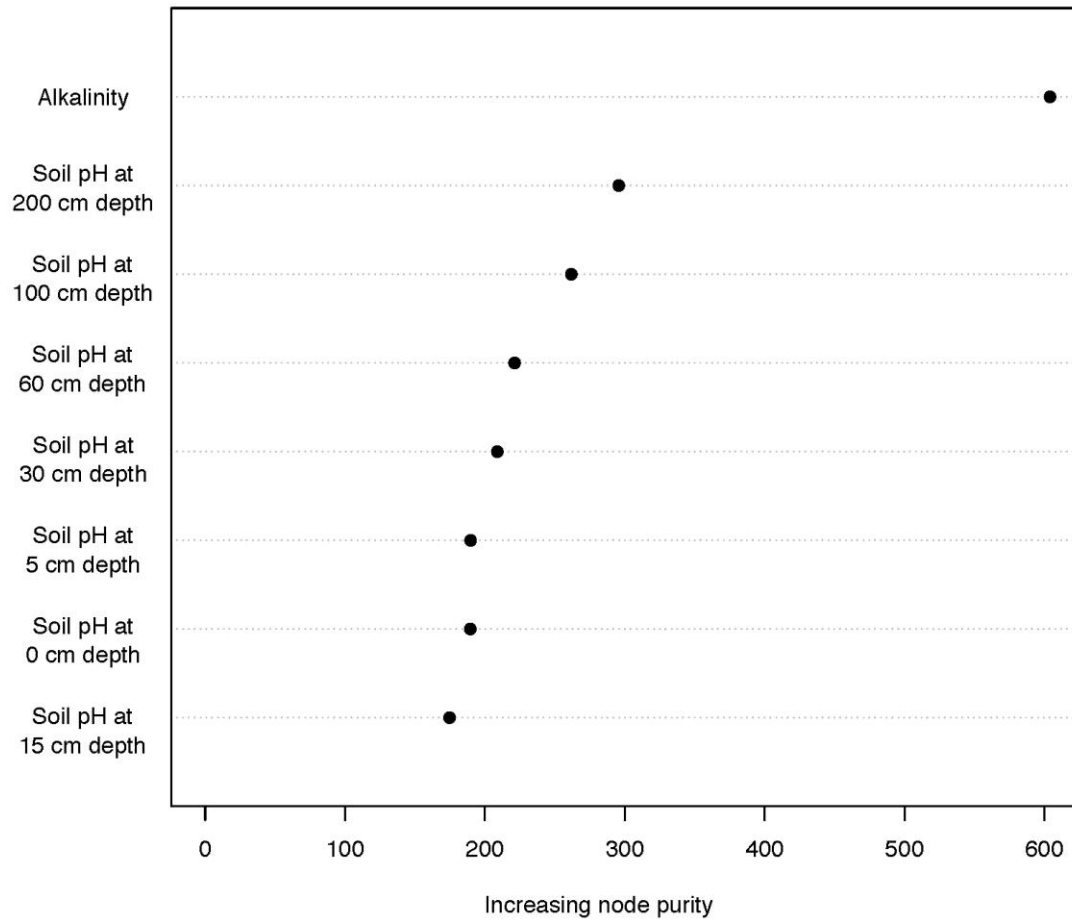
Variable importance plot of the Random Forest modelling global bicarbonate concentrations. Total increase in node purities from splitting on the variable, averaged over all trees and derived from residual sum of squares. The variables are ordered top-to-bottom as most-to-least important.

Fig. S6.
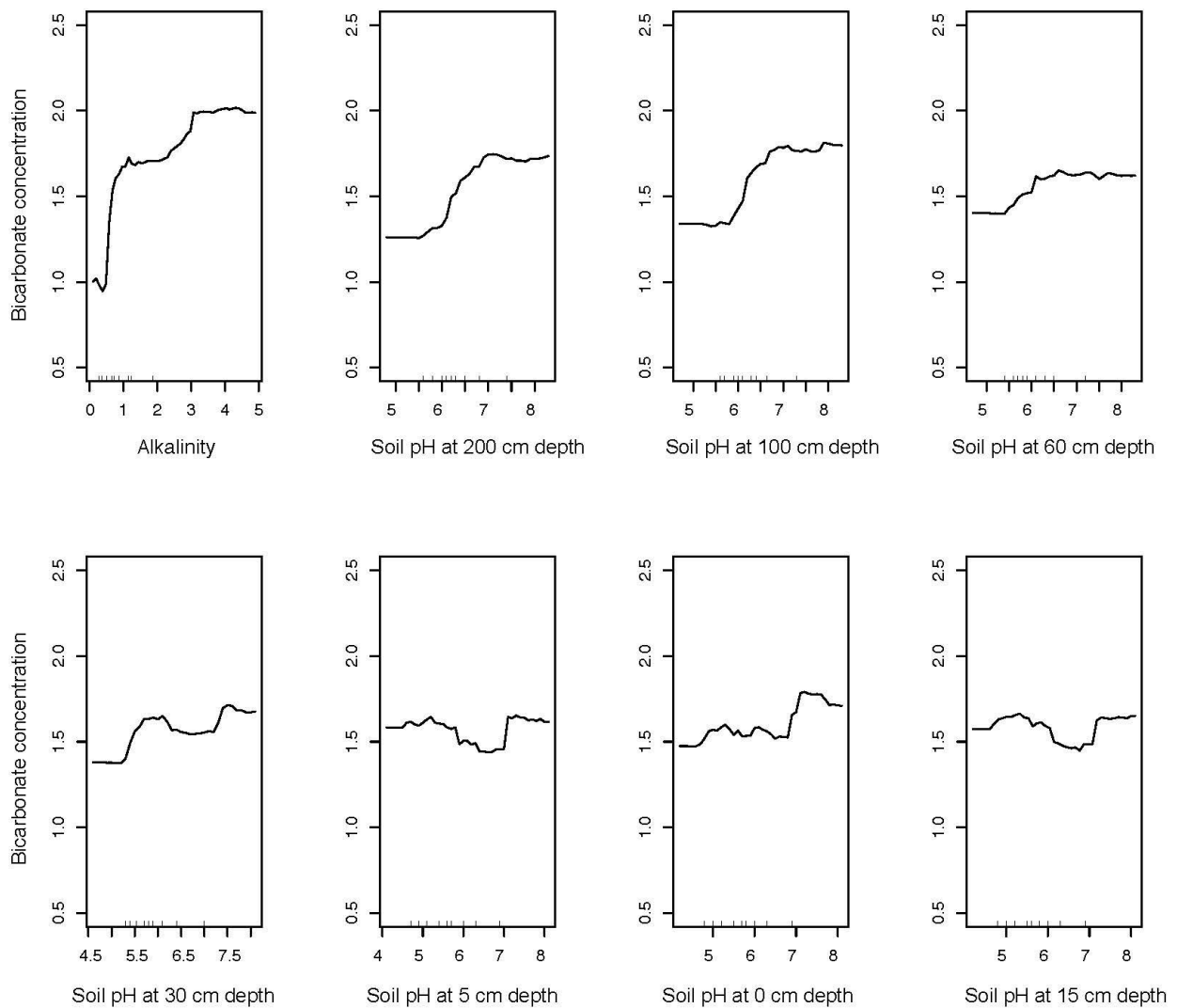
Partial dependence plots of the eight variables used to model global bicarbonate concentrations. Each plot represents the unique effect of the eight explanatory variables on global bicarbonate concentration. Relationships are derived form a collective Random Forest model (see Methods above).

Fig. S7.
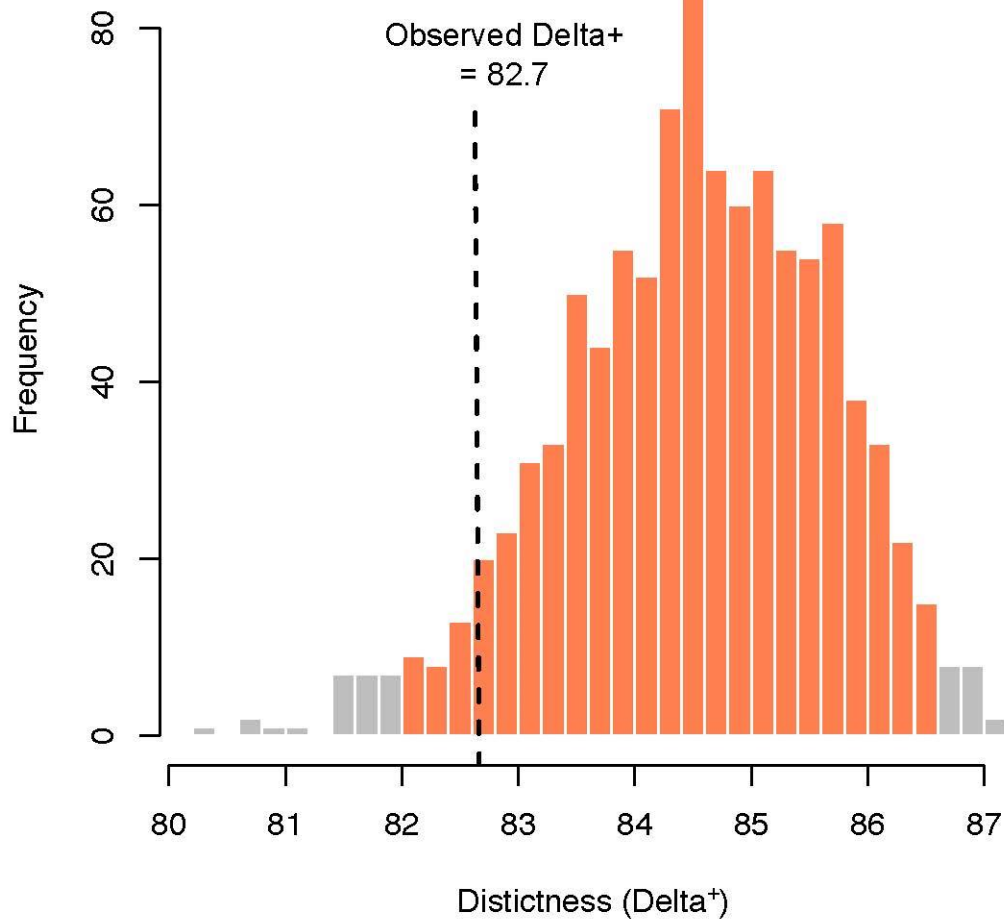
Histogram of taxonomic distinctness for 1000 random subsamples of a fixed number of 131 species drawn from a common species pool. The orange shaded areas depict the 95% quantiles of the 1000 random samples. The vertical dotted line shows the taxonomic distinctness value of the 131 study species.

Table S1.

Species	Trait	Source
Aldrovanda vesiculosa	CO_2	(*53*)
Alternanthera reineckii	CO_2	Original data
Aponogeton crispus	CO_2	Original data
Aponogeton longiplumulosus	CO_2	Original data
Aponogeton madagascariensis	CO_2	Original data
Aponogeton ulvaceus	CO_2	Original data
Berula erecta	CO_2	(*54*)
Blyxa aubertii	CO_2	Original data
Blyxa echinosperma	CO_2	(*55*)
Blyxa japonica	CO_2	(*55*)
Cabomba caroliniana	CO_2	(*55,56*)
Cabomba furcata	CO_2	Original data
Callitriche cophocarpa	CO_2	(*5,54*)
Callitriche hamulata	CO_2	(*53*)
Callitriche hermaphroditica	HCO_3^-	(*57*)
Callitriche longipedunculata	CO_2	(*58*)
Callitriche obtusangula	CO_2	Original data
Callitriche platycarpa	CO_2	Original data
Callitriche stagnalis	CO_2	(*54*)
Callitriche truncata	HCO_3^-	Original data
Callitriche verna	CO_2	Original data
Ceratophyllum demersum	HCO_3^-	(*4,55*)
Crassula aquatica	CO_2	(*58*)
Crassula helmsii	CO_2	(*59*)
Cryptocoryne albida	CO_2	Original data
Cryptocoryne crispatula	HCO_3^-	Original data
Cryptocoryne usteriana	CO_2	Original data
Deinostema violacea	CO_2	(*55*)
Echinodorus grisebachii	CO_2	Original data
Echinodorus palifolius	HCO_3^-	Original data
Egeria densa	HCO_3^-	(*55*)
Egeria najas	HCO_3^-	(*60*)

Elatine californica	CO_2	*(58)*
Eleocharis acicularis	CO_2	*(58)*
Elodea canadensis	HCO_3^-	*(61-63)*
Elodea nuttallii	HCO_3^-	*(55,64)*
Eriocaulon aquaticum	HCO_3^-	*(65,66)*
Eriocaulon decangulare	CO_2	*(67)*
Eriocaulon setaceum	CO_2	Original data
Gratiola aurea	CO_2	*(65)*
Helanthium tenellum	CO_2	Original data
Hippuris vulgaris	CO_2	*(35)*
Hydrilla verticillata	HCO_3^-	*(55,68,69)*
Hygrophila corymbosa	CO_2	Original data
Hygrophila polysperma	HCO_3^-	*(70)*
Isolepis fluitans	CO_2	*(71)*
Juncus bulbosus	CO_2	*(71)*
Lagarosiphon madagascariensis	CO_2	Original data
Lagarosiphon major	HCO_3^-	*(61)*
Lemna trisulca	HCO_3^-	*(71)*
Limnophila polystachya	CO_2	Original data
Limnophila sessiliflora	HCO_3^-	*(55,70)*
Littorella uniflora	CO_2	*(35,66)*
Lobelia dortmanna	CO_2	*(66,72)*
Ludwigia repens	CO_2	*(73)*
Luronium natans	HCO_3^-	*(74)*
Marsilea vestita	CO_2	*(58)*
Myosotis laxa	CO_2	*(54)*
Myosotis scorpioides	CO_2	*(54)*
Myriophyllum alterniflorum	HCO_3^-	*(4,35)*
Myriophyllum aquaticum	CO_2	*(55)*
Myriophyllum salsugineum	CO_2	*(75)*
Myriophyllum spicatum	HCO_3^-	*(5,35,55)*
Myriophyllum tuberculatum	HCO_3^-	Original data
Myriophyllum verticillatum	CO_2	*(5)*
Najas flexilis	CO_2	*(76)*

Najas indica	HCO_3^-	Original data
Najas marina	HCO_3^-	(*55*)
Najas oguraensis	HCO_3^-	(*55*)
Nechamandra alternifolia	HCO_3^-	(*55*)
Nuphar lutea	CO_2	(*35*)
Nuphar pumila	CO_2	(*55*)
Nymphaea lotus	CO_2	Original data
Nymphoides hydrophylla	HCO_3^-	Original data
Oenanthe aquatica	CO_2	Original data
Ottelia acuminata	HCO_3^-	(*77*)
Ottelia alismoides	HCO_3^-	(*77*)
Pogostemon helferi	HCO_3^-	Original data
Potamogeton acutifolius	CO_2	Original data
Potamogeton berchtoldii	HCO_3^-	(*71*)
Potamogeton crispus	HCO_3^-	(*14,35,55*)
Potamogeton distinctus	HCO_3^-	(*78*)
Potamogeton friesii	HCO_3^-	(*54*)
Potamogeton fryeri	CO_2	(*71,78*)
Potamogeton gramineus	HCO_3^-	(*79*)
Potamogeton lucens	HCO_3^-	(*55,73*)
Potamogeton maackianus	HCO_3^-	(*78*)
Potamogeton natans	CO_2	(*35,55,80*)
Potamogeton nodosus	HCO_3^-	(*78*)
Potamogeton obtusifolius	HCO_3^-	Original data
Potamogeton oxyphyllus	HCO_3^-	(*35,55*)
Potamogeton pectinatus	HCO_3^-	(*54,55*)
Potamogeton perfoliatus	HCO_3^-	(*35,54,55*)
Potamogeton polygonifolius	CO_2	(*35,81*)
Potamogeton pusillus	HCO_3^-	(*55,71*)
Potamogeton schweinfurthii	HCO_3^-	(*82*)
Potamogeton x angustifolius	HCO_3^-	(*14,35*)
Ranunculus aquatilis	HCO_3^-	(*14,35*)
Ranunculus circinatus	HCO_3^-	(*54*)
Ranunculus flabellaris	CO_2	(*83*)

Ranunculus fluitans	HCO$_3^-$	(*80*)
Ranunculus peltatus	HCO$_3^-$	(*14,63*)
Ranunculus penicillatus	HCO$_3^-$	(*71,84*)
Ranunculus trichophyllus	HCO$_3^-$	(*54*)
Rotala rotundifolia	CO$_2$	Original data
Rotala wallichii	CO$_2$	Original data
Sagittaria sagittifolia	CO$_2$	(*54*)
Sagittaria subulata	CO$_2$	Original data
Schoenoplectus subterminalis	HCO$_3^-$	(*85*)
Schoenoplectus torreyi	HCO$_3^-$	(*71*)
Sparganium emersum	CO$_2$	(*5,54*)
Sparganium erectum	CO$_2$	(*54*)
Stratiotes aloides	HCO$_3^-$	(*76,86*)
Stuckenia filiformis	HCO$_3^-$	(*35*)
Stuckenia pectinata	HCO$_3^-$	(*54*)
Subularia aquatica	CO$_2$	(*66,71*)
Utricularia australis	CO$_2$	(*53,87*)
Utricularia gibba	CO$_2$	Original data
Utricularia graminifolia	CO$_2$	Original data
Utricularia intermedia	CO$_2$	(*71*)
Utricularia minor	CO$_2$	(*53,87*)
Utricularia purpurea	CO$_2$	(*35,88*)
Utricularia vulgaris	CO$_2$	(*76*)
Vallisneria americana	HCO$_3^-$	(*71,89,90*)
Vallisneria nana	CO$_2$	Original data
Vallisneria natans	HCO$_3^-$	(*55*)
Vallisneria spinulosa	HCO$_3^-$	(*55*)
Vallisneria spiralis	HCO$_3^-$	(*5*)
Veronica anagallis-aquatica	CO$_2$	(*54*)
Veronica beccabunga	CO$_2$	(*54*)
Zannichellia palustris	HCO$_3^-$	(*71*)

List of freshwater angiosperms and their trait of inorganic carbon use. Species names were corroborated using The Plant List http://www.theplantlist.org/

Data S1. (separate file)

All R scripts and cleaned datasets used for this analysis are available at the Dryad Digital Repository.