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Contact CEH NORA team at
noraceh@ceh.ac.uk

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Adaptation by macrophytes to inorganic carbon down a river with naturally variable concentrations of CO₂

Maberly S.C.^{1,2}, Berthelot S.A.^{1,3}, Stott A.W.², Gontero B.¹

¹Aix-Marseille Université CNRS, BIP UMR 7281, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France

²Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP UK

³ Present address: Universitat Konstanz, Universitatstrasse 10, 78464, Konstanz, Germany

To whom correspondence should be addressed:

Stephen C. Maberly

Lake Ecosystems Group, Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP UK

email: scm@ceh.ac.uk

tel: +44 1524595851

Abstract

The productivity and ecological distribution of freshwater plants can be controlled by the availability of inorganic carbon in water despite the existence of different mechanisms to ameliorate this, such as the ability to use bicarbonate. Here we took advantage of a short, natural gradient of CO₂ concentration, against a background of very high and relatively constant concentration of bicarbonate, in a spring-fed river, to study the effect of variable concentration of CO₂ on the ability of freshwater plants to use bicarbonate. Plants close to the source, where the concentration of CO₂ was up to 24-times air equilibrium, were dominated by *Berula erecta*. pH-drift results and discrimination against ¹³C were consistent with this and the other species being restricted to CO₂ and unable to use the high concentration of bicarbonate. There was some indication from stable ¹³C data that *B. erecta* may have had access to atmospheric CO₂ at low water levels. In contrast, species downstream, where concentrations of CO₂ were only about 5-times air-equilibrium were almost exclusively able to use bicarbonate, based on pH-drift results. Discrimination against ¹³C was also consistent with bicarbonate being the main source of inorganic carbon for photosynthesis in these species. There was, therefore, a transect downstream from the source of increasing ability to use bicarbonate that closely matched the decreasing concentration of CO₂. This was produced largely by altered species composition, but partly by phenotypic changes in individual species.

Keywords: bicarbonate, Fontaine de Vaucluse, photosynthesis, river Sorgue, stable carbon isotope

Introduction

The function and biodiversity of inland waters is controlled by interactions between the physical and chemical environment and the physiological and biochemical acclimation and adaptation of the organisms present as well as their short-term behavioural responses. High biological demand for resources, created by high potential metabolic rates or high biomass density, or both, can exceed the rate of re-supply and so alter the chemical and physical conditions in inland waters. Conversely, uncoupling of biogeochemical cycles of production and decomposition can generate a high supply of resource when the demand is low.

The primary producers at the base of the aquatic food web can be planktonic, epiphytic or benthic. In rivers, benthic macrophytes, *Plantae* that are mainly derived from land plants that have re-invaded the water, are often dominant because the rapid water transit time can prevent the formation of large populations of phytoplankton, especially in upstream regions where nutrient concentrations can be low (Reynolds and Descy, 1996). Macrophytes can survive high flushing rates or low nutrient resources in the water, as in many upland rivers, because most have roots, anchoring them to the substrate and allowing them to access nutrients in the substrate.

However, freshwater macrophytes require high concentrations of CO_2 to saturate photosynthesis because of a high transport resistance across relatively large external boundary layers (Black et al., 1981, Maberly and Madsen, 1998). Photosynthesis can be limited further by intermittent depletion of CO_2 produced when rates of photosynthetic demand exceed rates of resupply and by the generation of high concentrations of oxygen that can promote photorespiration (Maberly and Madsen, 2002, Pedersen et al., 2013). However, aquatic photoautotrophs possess avoidance, exploitation and amelioration strategies to minimize the effects of potential carbon limitation (Klavnsen et al., 2011). Avoidance strategies involve living in environments where the concentration of CO_2 is naturally high, for example as a result of heterotrophic decomposition of organic matter (Maberly, 1985). Exploitation strategies include gaining access to more reliable sources of

CO₂ such as the atmosphere (Maberly and Madsen, 2002). Just over half of the freshwater macrophytes tested so far have amelioration strategies based on biophysical or biochemical carbon concentrating mechanisms (CCMs) to minimise carbon limitation photorespiration (Maberly and Madsen, 2002). The most widespread CCM is the use of bicarbonate as an alternative source of carbon. For biochemical details see also (Kroth, 2015) and Sage and Stata (2015). Because of physico-chemical equilibria, bicarbonate is less susceptible than CO₂ to depletion during removal of inorganic carbon by photosynthesis and is present at concentrations that exceed CO₂ at sites where the pH exceeds the pH representing the first pK value of the carbonate system at approximately pH 6.3.

Although the use of bicarbonate is widespread, it is not universal, probably because there are costs involved in its use. First, bicarbonate uptake involves moving bicarbonate against its electrochemical potential gradient and across an inherently impermeable plasmalemma and is therefore an active process requiring expenditure of ATP. In situations where light energy is low, species therefore tend to lack the ability to use bicarbonate. One example is the predominance of species at depth that lack an ability to use bicarbonate such as sublittoral marine red macroalgae (Maberly, 1990) and in freshwater, aquatic bryophytes (Krause-Jensen and Sand-Jensen, 1998). Secondly, there are further energetic costs associated with the production and maintenance of the proteins involved in bicarbonate use (Jones, 2005). Thirdly, there is also a 'permeability cost' involved in using bicarbonate: species that use bicarbonate have a lower affinity for CO₂ than species that are restricted to CO₂ (Maberly and Madsen, 1998). This is probably caused by a high internal resistance to CO₂ uptake (Madsen and Maberly, 2003) that minimises the diffusive loss of carbon actively pumped into the photosynthesising tissue, but consequently reduces rates of photosynthesis at limiting concentrations of CO₂.

Comparisons of the ability of freshwater macrophytes to use bicarbonate or operate a CCM are usually based on a comparison of plants collected from sites with very different physical and chemical characteristics. An alternative comparative approach compares the

physiology of plants or algae at sites with locally high concentrations of CO₂ with those at nearby sites with lower concentrations of CO₂. An example in the terrestrial environment includes studies at natural CO₂ vents associated with volcanic activity (Korner and Miglietta, 1994). An example from the marine environment is the use of geothermal vents to study the effects of high CO₂ and low pH as a surrogate for future ocean acidification (Hall-Spencer et al., 2008). This approach does not appear to have been used for freshwater macrophytes at a specific site despite the high variability in CO₂ concentrations in different surface waters and the frequently high CO₂ concentrations found in groundwater and groundwater-fed streams (Sand-Jensen and Staehr, 2012, Stets et al., 2009), although comparisons have been made over wider spatial areas e.g. (Demars and Tremolieres, 2009). The aim of the work reported here was to assess the ability of river macrophytes to use bicarbonate at different sites down a natural gradient of high CO₂ concentration from the groundwater-fed source of a river to lower concentrations downstream. The comparison is therefore made on plants experiencing very similar background physico-chemical conditions apart from CO₂. Furthermore, since this CO₂ gradient is likely to have been present for thousands of years, it allows adaptive responses to varying CO₂ to be distinguished from acclimative responses to short-term variability.

Materials and methods

La Sorgue at Fontaine de Vaucluse

Fontaine de Vaucluse, the source of the River Sorgue, is located in a Karst landscape in Provence, in south-east France (Fig. 1). It is the largest spring in France, and one of the largest in the world with a mean discharge of about $23 \text{ m}^3 \text{ s}^{-1}$ and minimum and maximum discharges of 4 and over $100 \text{ m}^3 \text{ s}^{-1}$ respectively (Bonacci, 2007). The recharge area comprises 1115 km^2 of Lower Carboniferous limestone, 1500 m thick, on largely uncultivated land dominated by Mediterranean forest and 'garrigue' - dry limestone scrubland (Blavoux et al., 1992) which on average receives an annual rainfall of 1096 mm (Bonacci, 2007). The outflow has a very high concentration of bicarbonate of about 4.25 mmol L^{-1} (Emblanch et al., 2003) with calcium as the dominant cation with a concentration of around 2 mmol L^{-1} (Garry et al., 2008). The geometric mean pH of 7.25, calculated from the frequent outflow data over a year presented in (Emblanch, Zuppi, 2003), was used to estimate an average CO_2 concentration of about 0.6 mmol L^{-1} assuming a water temperature of 12°C and an ionic strength of $0.0065 \text{ mol L}^{-1}$ based on ionic composition from adjacent cave water given in (Garry, Blondel, 2008) which had a similar bicarbonate concentration to the outflow.

Field methods

Samples were collected on 12/09/2013, between 11:00 and 16:00, down a transect from close to the source of the spring at Fontaine de Vaucluse to about 20 km downstream on one of the many distributaries in this region (Fig. 1). On this date, the discharge was relatively low at about $9 \text{ m}^3 \text{ s}^{-1}$ (REALPACA, 2013) which is slightly less than half the annual mean. At each site, water was collected in a large bucket and water temperature was measured immediately and the concentration of CO_2 measured with a Vaisala non-dispersive infrared gas analyser protected for use in water with a waterproof membrane as described by (Johnson et al., 2010). Water was stored in two completely-filled 50 mL Falcon polypropylene centrifuge tubes and kept in the dark in a cool box for analysis in the

laboratory on the next day. For stable carbon isotope measurement, 10 mL of lake or stream water was injected via a syringe into evacuated (< 5 Pa) 12 mL exetainer (Labco) containing 0.15 mL degassed concentrated phosphoric acid (Maberly et al., 2013, Waldron et al., 2007). Samples were collected in duplicate and kept inverted prior to analyses. At each site, macrophytes were collected by hand or with a drag rake and placed, moist, in polythene bags and stored in a cool box overnight.

Laboratory analyses

In the laboratory, macrophyte material for stable carbon isotope measurement was cleaned carefully and dried at 80°C for 24 hours and stored in aluminium foil before analysis. The pH of water from the river was measured with a combination pH-electrode (PHEL-GS2, Labbox) and meter (pH201, Hanna Instruments). Gran titration was used to measure alkalinity by duplicate titration of 15 mL samples with approximately 0.17 mol L^{-1} HCl that had been standardised against 1 mol L^{-1} NaHCO_3 (Mackereth et al., 1978). Inorganic carbon concentrations were calculated from measured temperature, pH and alkalinity following (Maberly, 1996). Calcite saturation was calculated as in (Maberly, 1996) using the equations of (Jacobson and Langmuir, 1974) to estimate the temperature-dependent calcite solubility product.

pH-drift experiments

Macrophytes and benthic filamentous algae were identified, cleaned carefully in tap water using a soft brush and rinsed in 1 mol L^{-1} NaHCO_3 for at least 20 minutes. This concentration was chosen because it has been frequently used and while high enough to allow sufficient bicarbonate, should it be present, it is not so high as to require extremely high pH values to be generated to deplete inorganic carbon. Three species from each site were placed in 13 mL of fresh 1 mmol L^{-1} NaHCO_3 in 15 mL Falcon tubes that contained about 2 mL of air. The

tubes were capped and placed horizontally in an incubator (Innova 4230, New Brunswick Scientific) at 17°C under continuous illumination from fluorescent tubes producing about 170 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (Macam Q201, Macam Photometrics, Livingstone, Scotland). pH was measured with the combination pH-electrode after 24 hours and roughly after every 12 hours until a maximum pH had been reached. Alkalinity was measured at the end of the experiment on duplicate aliquots by Gran titration.

Stable isotope analysis

For the analysis of inorganic carbon $\delta^{13}\text{C}$, four mL of helium (99.999%) was injected into the headspace of each exetainer to overpressurise. After shaking and 30 minutes equilibration a 40 μL gas sample was removed for analysis. $\delta^{13}\text{C}$ of the CO_2 was measured on an Isoprime Ltd Tracegas Preconcentrator coupled to an Isoprime Ltd Isoprime isotope ratio mass spectrometer. Pulses of known reference CO_2 and blanks were run prior to each batch.

Small amounts of the plant were re-dried at 105°C and aliquots sealed into 6 x 5 mm tin capsules and loaded into an autosampler (Eurovector Elemental Analyser, Eurovector, Milano Italy) coupled in-line to a stable isotope ratio mass spectrometer (Isoprime, Manchester, UK). Each sample was combusted at 1020°C with a pulse of oxygen and products were carried by a flow of helium through a reduction reactor containing copper wire at 650°C and dried with magnesium perchlorate. N_2 and CO_2 were separated by a packed GC column and delivered, via an 'open-split' to the isotope ratio mass spectrometer. Values were compared to pulses of CO_2 reference gas and to a solid working standard of known isotopic composition. Stable isotope methods for inorganic and organic C were accredited to UKAS ISO17025.

The $\delta^{13}\text{C}$ values of CO_2 and bicarbonate were calculated from the measured $\delta^{13}\text{C}$ of inorganic carbon and carbonate speciation using the temperature-dependent equations in (Mook et al., 1974) following (Maberly et al., 1992). Discrimination of plants against these

potential carbon sources was calculated as in (Maberly, Raven, 1992) and against atmospheric CO₂ with an assumed value of -8‰ (Verburg, 2007).

Results

The water temperature at the five sites varied by 2°C between 13.8°C at site A and 15.8°C at site E (data not shown). Over the first 8 km downstream from the source, represented by the first four sites, there was a large increase in pH from 7.35 to 8.05 (Fig. 2A). This was linked to a decrease in concentration of CO₂ from about 0.46 to 0.09 mmol L⁻¹ (24- to 5-times air-equilibrium; Fig. 2B). The CO₂ concentration declined with distance downstream over the first four sites, covering a distance of 6.6 km and a reduction in altitude of 15 m (i.e. a slope of 2.27 m km⁻¹), at about log_e 0.188 mmol L⁻¹ km⁻¹; R² = 0.96. Even at the fifth site, 20 km downstream from the source, the concentration of CO₂ was about five times the air-equilibrium concentration. These CO₂ concentrations were calculated from pH, alkalinity and temperature, but direct measurements in the field with the non-dispersive infra-red sensor gave very similar values and a comparison of the two methods across the five sites gave an R² of 0.94, but these data from the sensor are not used further. The alkalinity was high at all the sites and in contrast to the concentration of CO₂, its value only changed slightly from 4.84 to 4.73 mequiv L⁻¹ down the transect. The concentration of bicarbonate ions was correspondingly high and rather constant, varying between 4.83 and 4.68 mmol L⁻¹. The concentration of carbonate was only 0.005 mmol L⁻¹ at the source, but because of increasing pH, the concentration had increased to 0.028 mmol L⁻¹ (about 4.8-fold increase) at the fifth site. Assuming a calcium concentration of 2 mmol L⁻¹, calcite was about 1.4-times over-saturated at the source increasing to about 7-times over-saturated at the two lower sites (saturation index for calcite 0.15 to 0.85) and this may have been one of the reasons for the slightly lower alkalinity at the lower sites.

Nine species or genera of macrophytes and filamentous macroalgae were found at the five sites (Table 1). At the top of the transect, the vegetation was dominated by large underwater populations of *Berula erecta* (Fig. 3) along with the freshwater moss *Fontinalis*

antipyretica. At sites D and E with the lowest concentrations of CO₂ these were largely replaced by *Potamogeton nodosus*, *Stuckenia pectinata* (previously *Potamogeton pectinatus*) *Ceratophyllum demersum* and *Ranunculus fluitans* (Table 1).

All the species of macrophytes at the three sites nearest the source (sites A to C) had final CO₂ concentrations at the end of the pH-drift experiment greater than 1 µmol L⁻¹, raised the pH to less than 9.1 and had C_T/Alk quotients close to 1 (Table 2). The data are consistent with these species being restricted to CO₂ as a source of inorganic carbon for photosynthesis. The same was true for *B. erecta* at site D, but all the other species at this site showed evidence of bicarbonate use with pH values over 10.0 and final CO₂ concentrations that were between 20- and 150-times below the CO₂ compensation concentrations generated by the species restricted to CO₂. This was also the case for the species from the fifth site (site E). One species, *Ceratophyllum demersum*, however, showed signs of deterioration during the drift experiment and the final alkalinity at the end of the experiment was over twice the starting alkalinity; pH-drift data from this species are not used further.

The δ¹³C value of the inorganic carbon was relatively constant: it was most depleted at the source at -11.6‰ and varied between -10.5 and -10.9‰ at the four other sites- very similar to values reported in (Emblanch, Zuppi, 2003). Plant δ¹³C values were more negative (depleted) at sites with high concentrations of CO₂ (Fig. 4A). Similarly plant δ¹³C values were more negative for plants that were unable to use bicarbonate and hence had higher final CO₂ concentrations at the end of pH-drift experiments (Fig. 4B). The δ¹³C values for *Berula erecta* tended to be less negative than other plants that were restricted to CO₂.

Discrimination, Δ, was calculated for the different plant species for different putative sources of inorganic carbon (Fig. 5). The wholly submerged species that were restricted to CO₂ had an average discrimination value of 26.4 ‰ (SD = 1.4 ‰). If *B. erecta* was restricted to CO₂ in water its average discrimination value would be 15.5 ‰ (SD = 3.0 ‰). If instead its

organic carbon was produced solely from atmospheric CO₂, the average discrimination value would be 28.6 ‰ (SD = 3.2 ‰). For species with the ability to use bicarbonate, two potential forms of inorganic carbon are available. If these species were using CO₂, discrimination against CO₂ would be only 11.2 ‰ (SD = 2.7 ‰) but their discrimination against bicarbonate would be 21.6 ‰ (SD = 2.7 ‰).

There was a strong linear relationship between the final CO₂ in pH-drift experiments and the CO₂ concentration at the collection site (Fig. 6). The correlation was even stronger if *B. erecta* from site 4 was excluded from the data set ($R^2 = 0.99$; data not shown).

Discussion

Rivers generally have high concentrations of CO₂ because they receive large amounts of terrestrial organic carbon that has been degraded to inorganic carbon in the soil and have short residence times that limit evasion to the atmosphere (Cole et al., 2007, Raymond et al., 2013, Sand-Jensen and Staehr, 2012) although some rivers can be undersaturated with CO₂ during periods of high productivity (Neal et al., 1998). Groundwater-fed rivers often have particularly high concentrations of CO₂ at source because of sub-surface respiration and restricted exchange with the atmosphere (Cole, Prairie, 2007). In the case of the Fontaine de Vaucluse, data in (Emblanch, Zuppi, 2003) were used to calculate a mean concentration of CO₂ of 0.61 mmol L⁻¹ over a year at the source. On our sampling day, 0.3 km downstream from the source, the concentration was about 0.46 mmol CO₂ L⁻¹, corresponding to the mean in a compilation from groundwaters from New Zealand and Denmark (Sand-Jensen and Staehr, 2012) and similar to the maximum values reported in high alkalinity rivers in eastern France (Massabuau and Fritz, 1984). In limestone regions, these high concentrations of CO₂ are associated with high concentrations of bicarbonate, but high CO₂ associated with groundwater inputs can also be found in non-limestone regions where alkalinity is relatively low (Demars and Thiebaut, 2008).

Turbulent flow, especially in streams and rivers with steep slopes, leads to rapid reduction in the concentration of CO₂ by evasion to the atmosphere and net photosynthetic uptake causes the concentration to decline further (Butman and Raymond, 2011, Sand-Jensen and Staehr, 2012). This reduction in concentration of CO₂ was also found here; concentrations declined 2-fold over 3.7 km.

The species of macrophytes found in this study are typical of European rivers, especially those with a groundwater influence. For example, all the macrophytes found here were recorded in a survey of macrophytes from groundwater-fed regions of the rivers Rhine, Rhône and Moosach, (Demars and Tremolieres, 2009) apart from *P. nodosus*. Furthermore,

(Demars and Tremolieres, 2009) showed that plant distribution at different sites was related more to the concentration of CO₂ than to other chemical variables such as the concentration of ammonium or phosphate. We show here that, as previously assumed based on literature information, (Demars and Tremolieres, 2009), this pattern is caused by the differential ability of the different species to utilize bicarbonate as a source of inorganic carbon in addition to CO₂. At the first three sites below the spring down to about 3 km, the concentrations of CO₂ exceeded 10-times air-equilibrium (0.46 to 0.22 mmol L⁻¹) and all the species found were unable to use bicarbonate. The amphibious macrophyte *Berula erecta* was dominant at these sites and this species is known to be restricted to CO₂ (Sand-Jensen et al., 1992) and this was confirmed here. Based on our measurements of CO₂ concentration and the kinetic response of photosynthesis to CO₂ in (Sand-Jensen, Pedersen, 1992), we estimate that *B. erecta* would be between 75% and 100% saturated with CO₂ at these sites. At site D, where only small amounts of *B. erecta* were found, the CO₂ concentration of 0.09 mmol L⁻¹ would only support about 20% of the CO₂ saturated rates. The absence of species able to use bicarbonate at the upper sites, despite substantial concentrations of bicarbonate, exceeding 4.8 mmol L⁻¹, is consistent with substantial costs linked to the reduced affinity for CO₂ (Maberly and Madsen, 1998), and increased costs of running the process and producing and maintaining the extra machinery required (Jones, 2005). Conversely, at the lowermost sites, all species found and tested, had the ability to use bicarbonate. Here, CO₂ concentrations were only about 4.8-times greater than air-equilibrium which is likely to limit rates of photosynthesis if CO₂ was the only source of inorganic carbon (Sand-Jensen and Frost-Christensen, 1999).

The stable carbon isotope data can be used to give some indication of the source of inorganic carbon for the different species of macrophytes at the different sites if putative source values are known (Osmond et al., 1981). pH-drift experiments suggested that *F. antipyretica* and *Vaucheria* sp. were restricted to CO₂. Discrimination against CO₂ was about 25 to 28 ‰ which is consistent with typical C3 photosynthesis with little diffusion limitation

(Lloyd and Farquhar, 1994). Although *B. erecta* was also limited to CO₂, discrimination against this carbon source was only 12 to 19 ‰ which could indicate either diffusion limitation or that *B. erecta* may have taken up CO₂ from the atmosphere at very low water level even though the benefit is likely to be small at these very high CO₂ concentrations in the river as suggested by work on another amphibious species (Madsen and Breinholt, 1995). The discrimination values for species shown to use bicarbonate in the drift experiments were very low (8 to 16 ‰) against CO₂ but much more typical if expressed against bicarbonate (18 to 26 ‰) which may indicate that this was the dominant source of inorganic carbon even at moderate concentrations of CO₂.

B. erecta was found at four of the five sites, growing in CO₂ concentrations from 0.46 to 0.09 mmol L⁻¹. Although we do not have information on seasonal changes in CO₂ at the sites, the downstream gradient is likely to be relatively stable because the annual variation of discharge is relatively low and because the data in (Emblanch, Zuppi, 2003) show no significant correlation between CO₂ concentration at the source and discharge ($r = -0.039$). There was, however, some slight evidence for change in the CO₂ response of the different populations. In pH-drift experiments, the *B. erecta* population at the site nearest the spring had a final CO₂ concentration of about 19 µmol L⁻¹ whereas the three downstream populations sampled had final CO₂ concentrations of between 2 and 4 µmol L⁻¹. In contrast, the final CO₂ concentration of the aquatic moss, *F. antipyretica*, that was found at the three upper sites, was invariable at 2 to 3 µmol L⁻¹. More detailed work will be needed to determine whether phenotypic or genotypic change is involved in the putative differences in the *B. erecta* populations. Genotypic change is possible given the potentially large number of generations that could have occurred at this site over thousands of years. However, a study of soil algae at two high CO₂ (aerial) springs, found little evidence for genetic adaptation to high CO₂ concentrations (Collins and Bell, 2006). The ability of plants such as *B. erecta* to grow well in rivers is strongly linked to the high concentrations of CO₂ that can sometimes be found there (Sand-Jensen, Pedersen, 1992) and also explains why these species are

relatively rare in lakes which tend to have much lower CO₂ concentrations during most of the year (Baatrup-Pedersen et al., 2013). Although bicarbonate users can down-regulate their ability to use bicarbonate depending on inorganic carbon availability (Madsen et al., 1996) this does not appear to have allowed them to compete with *B. erecta* when concentrations of CO₂ were high at upstream sites.

Decomposition processes in the recharge zone can produce high concentrations of CO₂ in groundwater-fed rivers. This, coupled with a gradient of CO₂ loss downstream, produces a natural experiment to test the effect of elevated CO₂ on the biodiversity and physiology of aquatic plants in rivers which helps to explain the distribution of macrophytes in rivers.

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Figure legends

Fig. 1. Location of the sampling site in France (A), showing the catchment area (dashed line), the source (cross) and the downstream flow to the River Rhône (B) and the five sample sites A to E (C).

Fig. 2. Changes in carbonate chemistry on the River Sorgue with distance downstream from the source at Fontaine de Vaucluse. pH (A); alkalinity (open circles) and concentration of bicarbonate (closed circles; B); concentration of carbon dioxide measured directly (open circles) and calculated from pH, alkalinity and temperature (closed circles) with air-equilibrium indicated by a dashed line (C) and concentration of carbonate (D).

Fig. 3. The River Sorgue downstream from site A. Showing general appearance of the river (A) and the dominance of *Berula erecta* (B).

Fig. 4. Plant $\delta^{13}\text{C}$ values from the five sites for *Berula erecta* (open triangles), submerged CO_2 -users (open circles), and bicarbonate users (closed circles). A Plant $\delta^{13}\text{C}$ as a function of site CO_2 concentration; B Plant $\delta^{13}\text{C}$ as function of final CO_2 concentration in pH-drift experiments. The regression lines relate to all the combined data. Note the Log scale for concentration of CO_2 .

Fig. 5. Calculated discrimination, Δ , against air, (assumed -8‰; grey bars), carbon dioxide (white bars) and bicarbonate (black bars) for the different species on the River Sorgue below Fontaine de Vaucluse. Species are ordered by increasing ability to deplete inorganic carbon in pH-drift experiments. The asterisk indicates the likely source of inorganic carbon based on pH-drift experiments.

Fig. 6. Correlation between average final concentration of CO_2 at the end of a pH-drift experiment and the concentration of CO_2 at the sites from which the samples were collected.

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Table 1.

Species composition at the five sites on the River Sorgue (see Fig. 1 for location).

Species	A	B	C	D	E
<i>Berula erecta</i> (Huds.) Coville					
<i>Fontinalis antipyretica</i> Hedw.					
<i>Vaucheria</i> sp. DC.					
<i>Lemna trisulca</i> L.					
<i>Stuckenia pectinata</i> (L.) Böerner					
<i>Cladophora</i> sp. Kütz.					
<i>Ceratophyllum demersum</i> L.					
<i>Potamogeton nodosus</i> Poir.					
<i>Ranunculus fluitans</i> L.					

Table 2.

Mean carbonate equilibria from triplicate pH-drift experiments. Values are the maximal pH, the minimal concentration of total inorganic carbon (C_T) and CO_2 and the quotient of C_T /alkalinity.

Site	Species	pH	C_T (mmol L ⁻¹)	CO_2 (mmol L ⁻¹)	C_T /Alk
A	<i>Berula erecta</i>	7.61	0.348	1.98E-02	1.06
A	<i>Fontinalis antipyretica</i>	9.02	0.960	2.16E-03	0.95
B	<i>Berula erecta</i>	8.55	0.324	2.20E-03	0.99
B	<i>Fontinalis antipyretica</i>	8.91	0.971	2.81E-03	0.96
B	<i>Vaucheria</i> sp.	8.54	1.174	8.16E-03	0.99
C	<i>Berula erecta</i>	8.34	0.328	3.57E-03	1.00
C	<i>Fontinalis antipyretica</i>	9.05	0.957	1.99E-03	0.95
D	<i>Berula erecta</i>	8.29	0.328	4.03E-03	1.00
D	<i>Cladophora</i> sp.	10.73	0.458	6.33E-06	0.42
D	<i>Lemna trisulca</i>	10.31	0.734	4.61E-05	0.61
D	<i>Stuckenia pectinata</i>	10.43	0.339	1.62E-05	0.49
E	<i>Ceratophyllum demersum</i>	9.72	2.266	9.10E-04	0.81
E	<i>Potamogeton nodosus</i>	10.27	0.805	6.99E-05	0.60
E	<i>Ranunculus fluitans</i>	10.45	0.294	1.19E-05	0.48

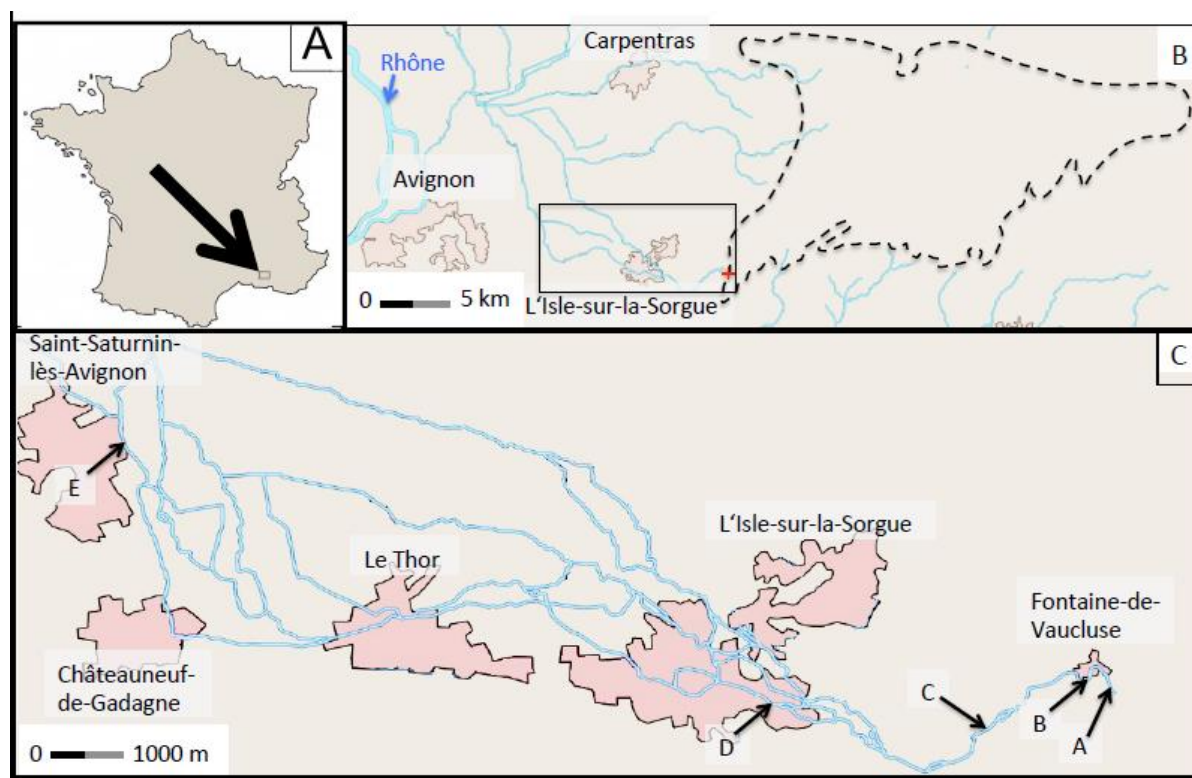


Fig. 1.

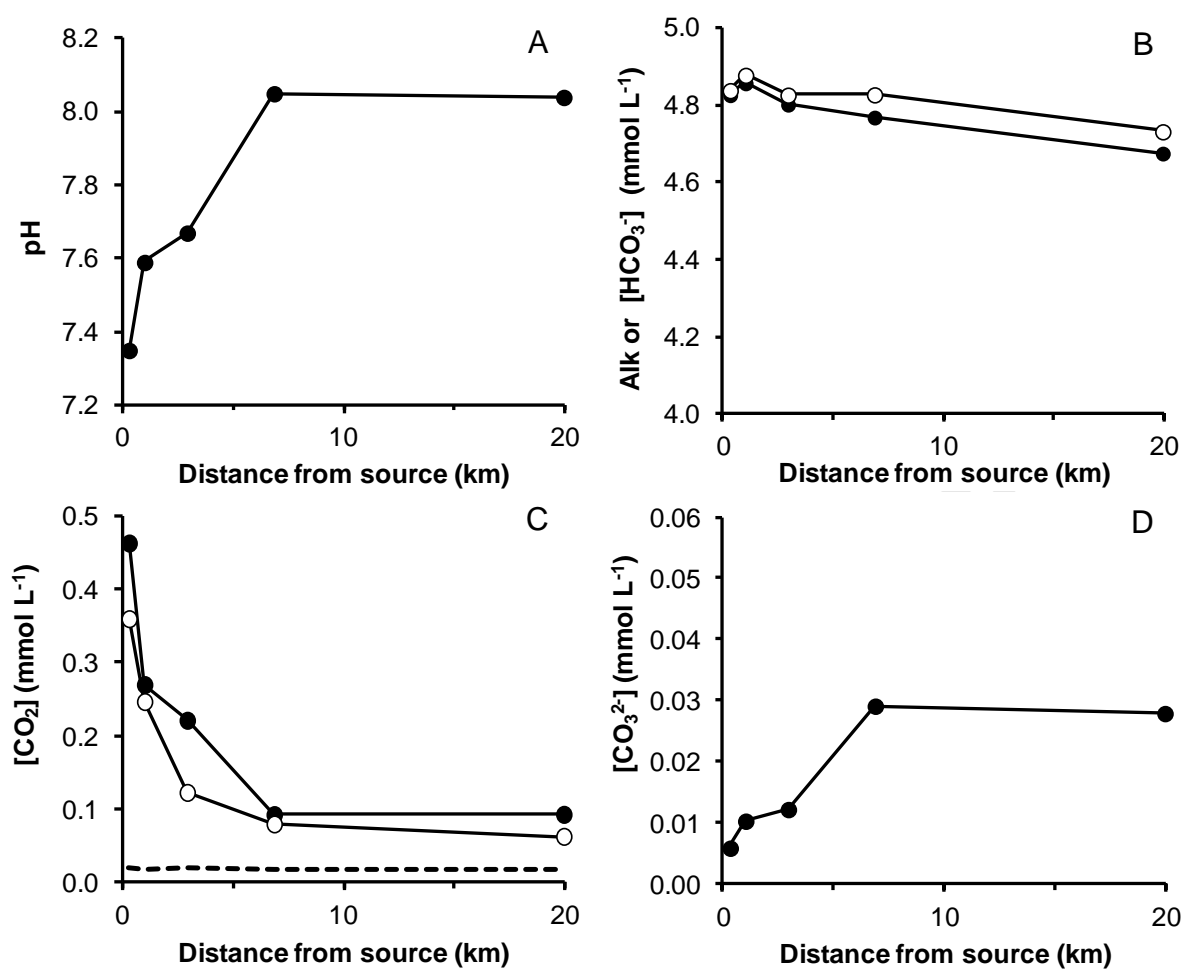


Fig. 2



Fig. 3

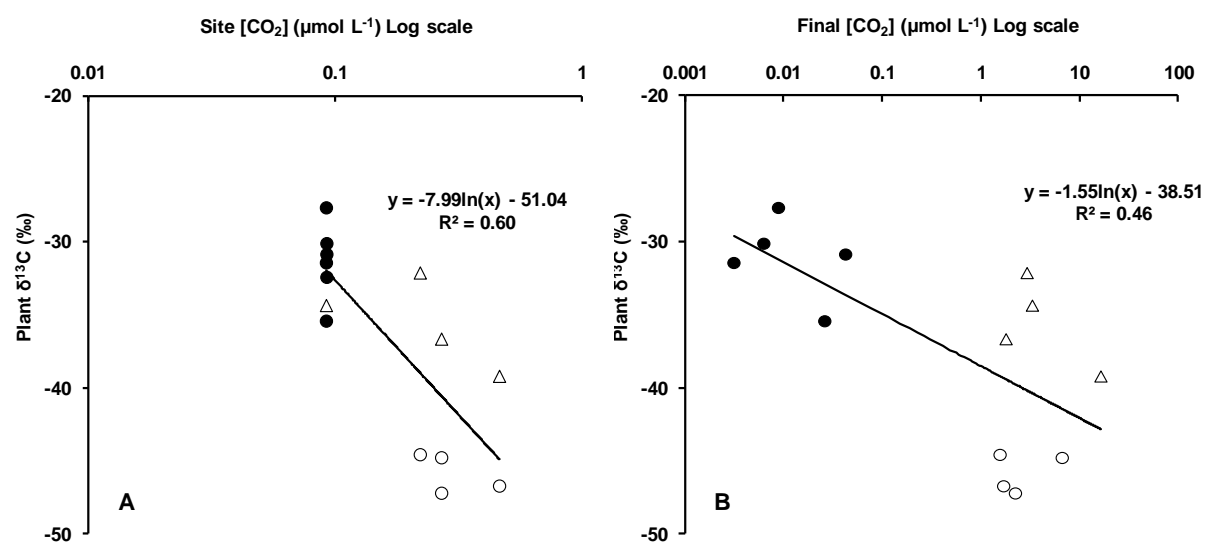


Fig. 4

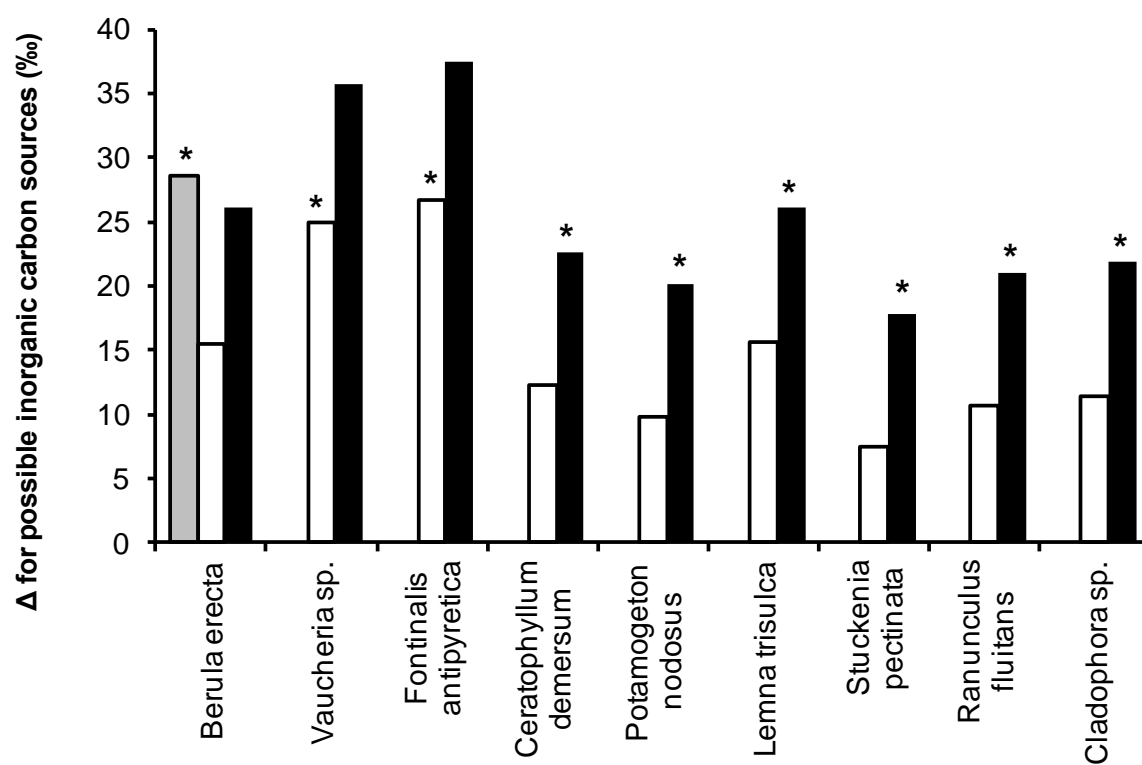


Fig. 5

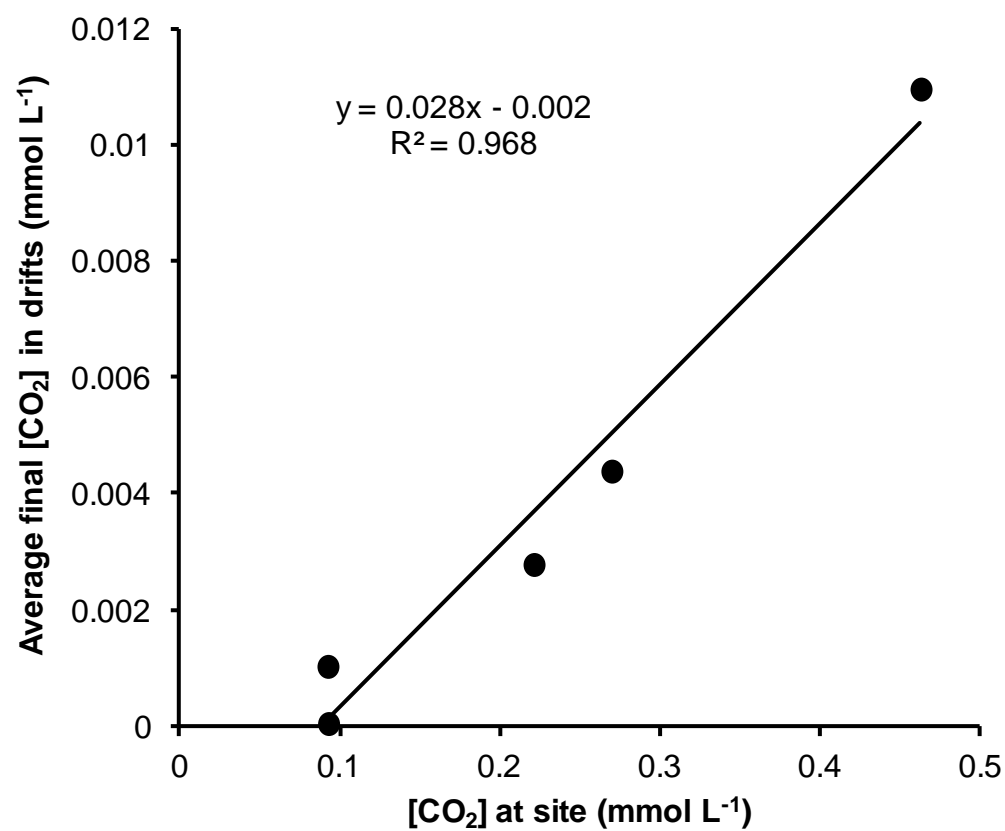


Fig. 6