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# Cyanobacteria–phosphate–calcite interactions in limestone (hardwater) streams of England

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**Abstract** Hardwater streams often contain deposits of calcium carbonate (travertine, tufa) supporting well-defined communities of cyanobacteria. Deposition of carbonate crusts is inhibited by moderate levels (c.100 µg/l) of dissolved orthophosphate phosphorus that can result in their loss, along with their cyanobacteria. Thirteen English cyanobacterium crust communities were investigated and in all but one, orthophosphate levels were too low to influence carbonate deposition. At one site, inhibitory levels of orthophosphate were found, and here, crusts have not been recorded for over 40 years. Phosphorus was detected in both the inorganic ($CaCO_3$) and organic fractions of the crusts, and in the latter, it was highest in the living cyanobacterium mat. No interaction between the phosphorus in these two fractions was observed. Levels of total phosphorus in the crusts were positively correlated with orthophosphate in the water. The great majority of English lowland waters currently contain inhibitory levels of dissolved orthophosphate preventing the development of these communities over large tracts of the country. Estimates of the uptake of phosphorus by the cyanobacteria suggested that their growth would not significantly influence the inhibition of precipitation of the associated calcium carbonate.

**Keywords** Calcium carbonate · Precipitation · Inhibition · Orthophosphate · Statistics · *Phormidium*

## Introduction

It was Fritsch & Pantin (1946) who first described in detail the close association between freshwater calcareous deposits and cyanobacteria in the British Isles, when the long drought of 1943 exposed deposits in a small Cambridgeshire stream. They identified the principal organism as *Phormidium incrustatum* (Naeg.) Gomont and drew attention to similar deposits described from the eastern United States (Roddy, 1915). Fritsch extended his search and found similar deposits in a further four English chalk streams (Fritsch, 1949, 1950) always associated with the *Phormidium* and sometimes with *Schizothrix fasciculata* (Naeg.) Gomont. At a nearby location on the River Ivel, Edwards & Heywood (1960) found snails heavily encrusted with calcareous nodules above a sewage treatment works but could find no crusts below, but they did not record any associated cyanobacteria. No further investigations were undertaken until the author began his PhD in 1971. Subsequently, many further sites for these deposits have been found in the headwaters of limestone

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streams (Pentecost, 2003). During these more recent surveys, it became clear that most of the sites reported by Fritsch no longer supported incrustations. Two causes were suggested for their demise: dredging and inhibition of calcite precipitation by dissolved phosphates. The second of these is the subject of this paper.

Laboratory studies have demonstrated that calcite nucleation and crystal growth are inhibited by orthophosphates and related oxy-phosphorus compounds at low concentration (Meyer, 1984). These studies have often been industry-led and have resulted in the development of specific phosphate inhibitors for the prevention of scale formation in industrial processes and water supply (Amjad, 1995). Providing that phosphorus concentrations are not excessive, carbonates can remove it from fresh waters, reducing the potential for eutrophication (Koschel, 1997). Much of the phosphorus is believed to be removed through co-precipitation with calcite and this may reduce dissolved phosphorus concentration to the point where it may become limiting for algal growth (Noe et al., 2001; Corman et al., 2016a). There have been several studies of phosphorus dynamics in streams depositing calcium carbonate. Corman et al. (2016b) found that epilithon biomass P-content increased in a shaded stream where calcium carbonate deposition had declined due to a reduction in photosynthesis. In Eastern Europe, Kepcija et al. (2011) found that higher P levels in the water led to more carbonate deposition that may be linked to increased periphyton photosynthesis. Both studies suggest a direct effect of calcium carbonate deposition on the uptake of P by the algae. Other studies indicate that trapped allochthonous carbon, common in wooded catchments, may decay and hence release its phosphorus more readily if carbonate encrusted (Belančić et al., 2009) although it may be less attractive to shredders (Martinez et al., 2015). The loss of carbonate precipitation in the natural environment is also of concern (Pentecost, 2005). In streams, travertine deposits provide a large surface area often colonised by a specialist flora and fauna, adding to freshwater diversity (Durrenfeldt, 1978; Boyer & Wheeler, 1989). Travertine dam formation will also increase the water capacity of a stream system and may help alleviate flooding.

Cyanobacteria that colonise travertine also have a phosphorus requirement and this must be acquired from their immediate environment. Thus the interaction between cyanobacteria, calcite deposits and phosphorus is a topic worthy of investigation.

In this article, evidence for phosphate inhibition of calcite precipitation is presented and the values compared with current estimated orthophosphate levels in English streams and rivers. Although representing a small area of the earth's surface, England has a detailed record of phosphate–phosphorus levels in its waters and its travertine-depositing sites are well known. The country also includes contrasting areas of human population, topography and geology thus providing a wide range of potential depositing environments.

## Methods

### Sampling sites

Thirteen sites were chosen for the collection of carbonate-encrusted cyanobacteria, plus samples of spring and stream water. Site locations and descriptions are given in Table 1 and Figs. 1, 2. All sites were located in England with groundwater emerging from the English Chalk or the Carboniferous Limestone Formation.

### Cyanobacteria

Surface scrapes of carbonate crusts were immersed in 3.5% disodium ethylenediamine tetraacetic acid ($Na_2EDTA$) pH 8 for 24 h to remove calcium carbonate. Samples were then washed in water and examined on glass slides in transmitted light with a Meiji Techno microscope at magnification of 125–750 diameters. Cyanobacteria were identified using Komarek & Anagnostidis (2005) and Whitton (2011). Other algae, if present, were identified only to genus. The relative abundance of cyanobacteria was assessed using the AFOR scale (abundant, frequent, occasional, rare).

### Water chemistry

Water samples were collected from the locations at which the algal crusts were taken and from the feeder springs. The distance between these locations ranged from 40 to 250 m. Water temperature was determined

**Table 1** Sampling site locations and descriptions

| Site name | Code | Lat/long | Month/year sampled | Altitude m | Water temperature (°C) | PH | Ca mM/l | Calcite sat. quotient | Geology |
|---|---|---|---|---|---|---|---|---|---|
| Borne Brook, Cambridge | 1 | 52.11N, 0.04E | March 2017 | 12 | 10.0 | 7.77 | 4.27 | 6.16 | C |
| Brigsteer, Cumbria | 2 | 54.18, 2.47W | April 2016 | 55 | 9.8 | 8.25 | 2.22 | 9.00 | CL |
| Conistone, N. Yorks | 3 | 54.06N, 2.02W | May 2016 | 200 | 10.8 | 8.91 | 1.80 | 5.76 | CL |
| Cunswick, Cumbria | 4 | 54.20N, 2.46W | April 2016 | 130 | 8.2 | 8.26 | 2.20 | 8.67 | CL |
| Ingleton, N. Yorks | 5 | 54.10N, 2.27W | May 2016 | 160 | 9.3 | 8.78 | 1.23 | 4.27 | CL |
| Natland 1, Cumbria | 6 | 54.17N, 2.45W | May 2016 | 30 | 14.0 | 8.88 | 2.22 | 14.7 | CL |
| Natland 2, Cumbria | 7 | 54.17N, 2.45W | May 2016 | 30 | 15.4 | 8.94 | 2.00 | 14.3 | CL |
| Malham Tarn, N. Yorks | 8 | 54.05N, 2.10W | April 2016 | 340 | 8.0 | 9.05 | 1.16 | 5.71 | CL |
| Mastiles West, N. Yorks | 9 | 54.05, 2.08W | April 2016 | 360 | 9.1 | 8.69 | 2.41 | 8.24 | CL |
| Sleets Gill, N. Yorks | 10 | 54.07N, 2.03W | May 2016 | 240 | 11.8 | 8.62 | 1.60 | 4.81 | CL |
| Throstle's Nest, N. Yorks | 11 | 54.07N, 2.02W | May 2016 | 210 | 12.2 | 9.08 | 1.24 | 6.00 | CL |
| Waterfall Beck, N. Yorks | 12 | 54.07N, 2.08W | April 2016 | 430 | 6.0 | 8.76 | 1.60 | 4.38 | CL |
| Whitbarrow, Cumbria | 13 | 54.15N, 2.50W | June 2016 | 90 | 12.3 | 8.86 | 1.55 | 5.79 | CL |

Measurements of temperature, pH and dissolved Ca refer to the crust locations. Under geology, *CL* carboniferous limestone formation, *C* chalk

in situ with an Edale D 515 thermistor and water samples returned to the laboratory in iodised polythene bottles. Operational dissolved orthophosphate (OP) and total dissolved orthophosphate (TP) was determined with the methods described in Mackereth et al. (1978). Samples were analysed within 5 h of collection and distilled water blanks were included. Water pH was determined on duplicate samples with an Orion 250A pH meter and dissolved calcium determined using a compleximetric titration (Mackereth et al., 1978). Calcite saturation quotients were determined using an iterative method (Pentecost, 1992). Statistical analyses were performed using Minitab 10 for Windows.

## Analysis of carbonate crusts

The surface layer containing living cyanobacteria (≤2.5 mm deep, c.5 $cm^2$ area) was removed using a small diamond drill, transferred to a glass vial and dried at 105 °C for 2 h. Samples were ground in a pestle and mortar and passed through a 250-µm stainless steel sieve and weighed. Powders were then transferred to PTFE beakers to which 5-ml ice-cold N HCl was added to dissolve the carbonate. After 30 min, the suspension was filtered, weighed and dried onto 1-µm pore size GFC glass fibre filters. The filtrate was neutralised with N NaOH then made up to 25 ml with water. A 15 ml aliquot was removed for P analysis and the remaining 10 ml oxidised with acid persulphate to determine the total P-content. By finding the difference between the two analyses, the amount of dissolved organic P could be estimated. The acid-insoluble residue held on the filter (remains of algae and detritus) was dried at 105 °C for 2 h, re-weighed then placed in porcelain crucibles and ignited in a furnace at 550 °C for 2 h. The ignited filters were then re-weighed and placed in beakers with 2.5 ml N HCl for 30 min at room temperature. The solution was removed with a pipette, the filters washed twice with distilled water and the combined solution made up to 10 ml after neutralisation with N NaOH. Samples were then analysed for OP. The phosphorus concentration in this fraction represents that present in the acid-insoluble organic matter present within the crusts plus some P that may have been released from detrital acid-insoluble non-carbonate minerals in the crust.

The above method was also applied to crusts 2.5–5 mm below the surface, when they were present. In addition, a longer profile was obtained from a thicker crust at site 5 to a depth of 25 mm. In this case, the drilled samples were taken every 2.5 mm, ground, dried and weighed as before but incinerated directly to obtain an integrated P analysis only. In all cases, two blanks were run through the entire procedure.

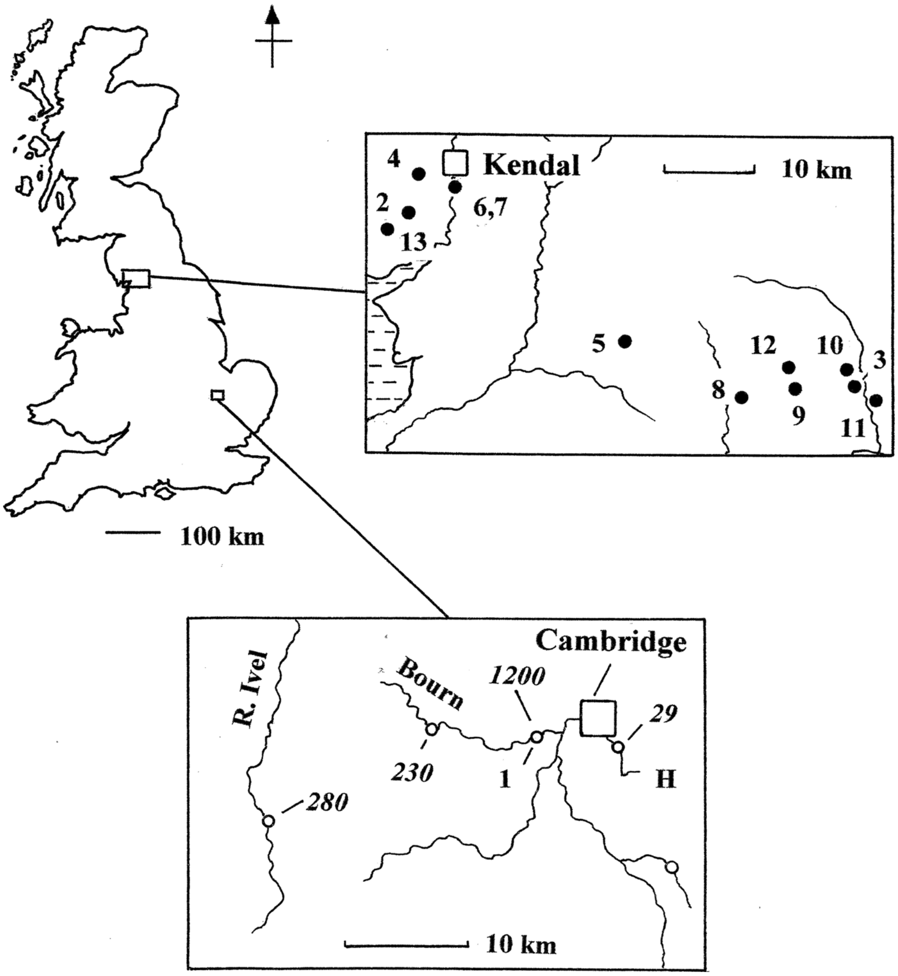

**Fig. 1** Locations of the sampling sites for carbonate crusts, cyanobacteria and water chemistry. Site 1 is shown as an *open circle* in the *inset*. *Open circles* represent sites formerly known for *Phormidium* crusts but now lost. Current (July, 2016) dissolved orthophosphate levels in µg/l are shown for some these sites in *italics*

## Results

### Cyanobacteria

*Phormidium incrustatum* (Naeg.) Gomont was the most abundant cyanobacterium encountered, occurring in ten out of the thirteen samples, sometimes to the exclusion of other species (Table 2). The narrower *P.* cf. *ingrediens* Backhaus was often associated, as were *Schizothrix*-like forms with narrow (0.8–1.8 µm) trichomes but usually lacking the characteristic compound sheaths. Coccoid forms were often present but rarely in large numbers. Heterocystous forms were observed at six of the sites, notably 8 (Malham Tarn) and 9 (Mastiles) both of which were dominated by members of the Rivulariaceae. *Capsosira brebissonii* Kuetz. (Stigonematales) occurred occasionally among the Rivulariaceae at site 8.

### Phosphate–phosphorus in water and crusts

Concentrations of phosphorus as TP and OP in the water at both spring and crust sites are shown in Fig. 3. In all but one case, concentrations at both spring and crust sites were below 10 µg/l and in twelve cases, levels fell below the limit of detection (1 µg/l). A two-way analysis of variance demonstrated that overall there was no significant difference between levels at

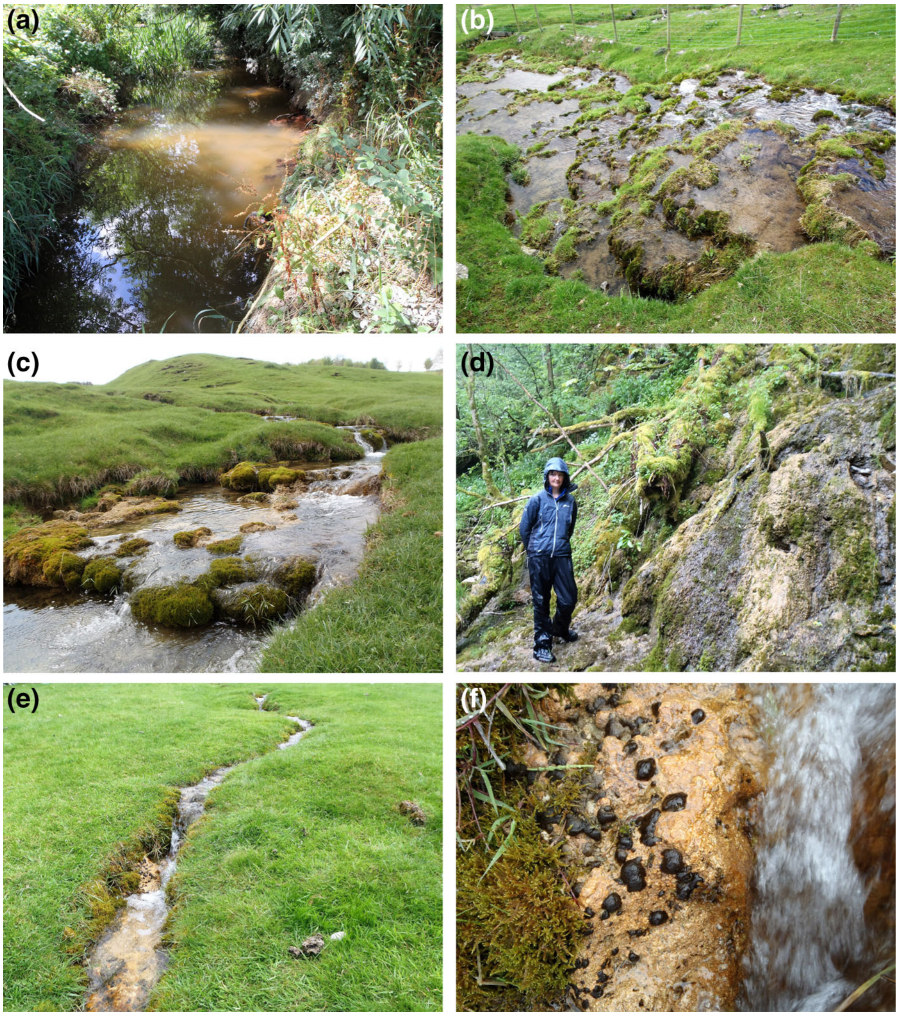

**Fig. 2** Images of five of the crust sampling sites. **a** Site 1, the Bourn Brook near Cambridge once known for *Phormidium* incrustations. **b** Site 2, Brigsteer, showing mossy travertine dams colonised by *Phormidium incrustatum.* **c** Site 3, Conistone springs with mossy travertine dams colonised by *Phormidium* spp. **d** Site 5 Ingleton travertine cascade colonised by bryophytes and *Phormidium incrustatum.* **e** Site 11, Throstles Nest Stream. **f** Detail of crusts with uncalcified mats of *Tolypothrix tenuis (dark)* and calcified *Schizothrix (pink)*

the crust sites and the feeder springs in terms of either OP or TP ($F = 0.02$, $P = 0.90$; $F = 1.04$, $P = 0.33$, respectively, variances homoscedastic) but there was considerable variability both between and within sites. Also the relative proportion of OP to TP varied both within and between sites. In almost every case, there were detectable levels of dissolved organic orthophosphate (TP-OP). The ratio between dissolved organic P and OP showed much variation but on average, dissolved organic P was 53% of TP so that OP was 47% of TP. The calcite saturation quotients at all sites exceeded unity and averaged 7.52 (Table 1).

Phosphorus was found in all of the crusts (Fig. 4). The amount detected was variable, the highest concentration being found at site 1. This is one of Fritsch's original sites near Cambridge where deposits no longer occur (Fig. 2a). Total P values in the surface crusts (left-hand bars in Fig. 4) ranged from 109 to 1038 ppm and averaged 420 ppm (Table 3). Phosphorus associated with the mineral fraction, which averaged 97.5% calcite, averaged 201 ppm while that associated with organic matter averaged 210 ppm. Phosphorus levels in these surface crusts (0–2.5 mm depth) contained the bulk of the live algae and

**Table 2** Cyanobacteria recorded in the crusts as relative abundance

| Site | *Aphanocapsa* spp | *Gloeocapsa punctata* | *Hydrococcus rivularis* | *Hyella fontana* | *Phormidium incrustatum* | *Phormidium cf ingrediens* | *Schizothrix*-like forms | *Calothrix* spp | *Dichothrix* spp | *Rivularia* spp | *Tolypothrix tenuis* | *Capsosira brebissonii* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | **** | | * | | | | | |
| 2 | * | | | ** | *** | *** | ** | | | | | |
| 3 | | | * | * | * | *** | | | | | | |
| 4 | *** | | | * | ** | | * | | | | | |
| 5 | | | | | **** | * | | | | | | |
| 6 | | | | | *** | | | | | | | |
| 7 | | | | | ** | *** | ** | | | | * | |
| 8 | ** | | | | | | ** | ** | **** | | | ** |
| 9 | | | | | | | | | | **** | | |
| 10 | * | * | | * | **** | *** | * | | | | | |
| 11 | * | * | | | | * | *** | *** | | | ** | |
| 12 | | * | | | *** | | *** | * | | | | |
| 13 | * | ** | | | **** | * | * | | | ** | | |

Abundant ****; frequent to common ***; occasional **; rare *

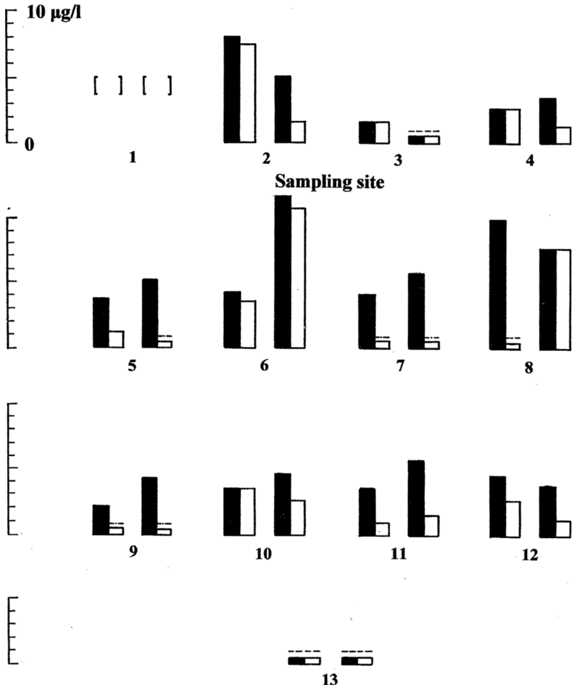

**Fig. 3** Boxplots showing concentration of total dissolved orthophosphate (*solid*) and dissolved orthophosphate (*open*) for each site. *Boxes* on the *left* show concentration at the springhead, those on the *right* at the crust sampling sites. Values below the level of detection are shown with a *horizontal broken line*. There are no historic values available for site 1

cyanobacteria as evidenced by their pale bluish-green colouration. Levels in the layer immediately below (2.5–5 mm depth) are also shown in Fig. 4 and Table 3. There was considerable variation between the sites but a two-way analysis of variance showed that levels of P in the organic fraction at the surface were significantly higher than those in the layer immediately below ($F = 24.3$, $P < 0.001$; variances homoscedastic) while the levels in the calcite fraction were not significantly different ($F = 0.85$, $P = 0.38$). A further series of total P analyses conducted on material from site 5 (Fig. 5) further indicate maximum levels at the surface and lower but variable levels down to a depth of 2.5 cm below the surface. There was a positive but weak correlation between the OP levels in the water and P levels in the calcite fraction of the surface crusts ($r = 0.45$, $P = 0.13$) but no significant correlations were obtained between the P levels in the water (TP or OP) and the surface organic fraction, surface crust or the surface crust acid-insoluble mineral residue.

## Discussion

### Cyanobacteria

Cyanobacteria associated with carbonate stream crusts have been well studied (Golubic & Fischer, 1975; Freytet & Plet, 1996; Pentecost & Whitton, 2000) and have been found to be more or less cosmopolitan. Most abundant and widespread among them is *P. incrustatum* whose global distribution has been described (Pentecost, 2003). This simple filamentous cyanobacterium usually forms a thin layer up to 2.5 mm in depth within accreting carbonate crusts influencing their formation by providing a sticky mucilaginous surface capable of trapping and binding small carbonate particles. It also assists in calcite precipitation through its photosynthesis (Pentecost, 2003).

### Phosphorus in water and crusts

Data on phosphate–phosphorus concentrations in the headwaters of calcareous streams are limited. They tend not to be regularly monitored by the state and are only sampled in cases of gross pollution or by specialist researchers. In regions of low population density and land management, dissolved orthophosphate levels tend to be low owing to the limited solubility of the principal phosphorus-containing mineral phase, apatite. Concentrations in ground waters rarely exceed 30 µg/l at circumneutral pH (Stumm & Morgan, 1996). This is the range in which most clean calcium-rich spring waters lie in both England (e.g. Pentecost, 1992; Ellwood & Whitton, 2007) and continental Europe (Kempe & Emeis, 1985; Hägele et al., 2006). Dissolved orthophosphate is but a fraction of the total dissolved P and in this study that fraction ranged from 1 to 74%, averaging 53%. In a study of three travertine-depositing streams in Arizona, Corman et al. (2015) found that the stream averages for the dissolved orthophosphate fraction ranged from 21 to 44%. The organic phosphate fraction is believed to be made up of dissolved and/or colloidal organic phosphorus compounds, some of which may be utilised by cyanobacteria using alkaline phosphatase (Whitton & Neal, 2011).

Crusts contained significant levels of phosphorus and the fractionation indicated that it was divided primarily into an organic and inorganic (calcium carbonate, calcite) fraction. It was hypothesised that as

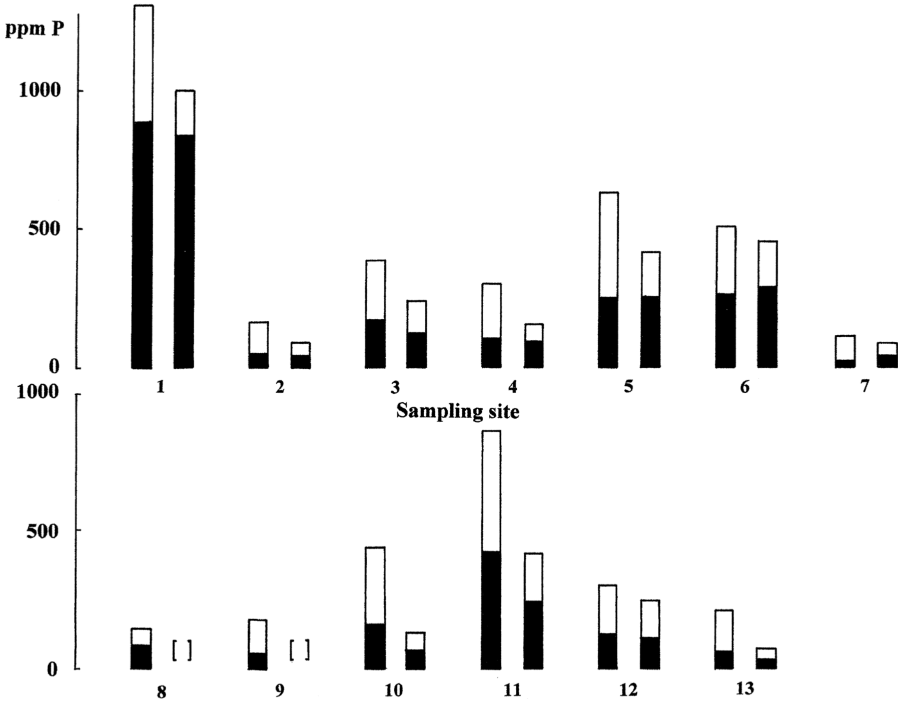

**Fig. 4** Phosphorus concentrations in the stream crusts shown as boxplots. For each site, the *left-hand bar* represents the surface layer (0–2.5 mm) and the *right-hand bar* the layer immediately beneath it (2.5–5 mm). Phosphorus associated with calcium carbonate (*black*), phosphorus associated with organic matter (*white*)

**Table 3** Mean levels of phosphorus in the carbonate crusts with ranges in parentheses

| | Surface 0–2.5 mm | Underlayer 2.5–5 mm |
|---|---|---|
| Total P ppm | 420 (109–1308) | 278 (68–991) |
| Calcite P | 201 (17–882) | 173 (32–831) |
| Organic P | 219 (47–432) | 105 (36–174) |

the cyanobacteria decayed through burial by the accreting crust, the released P would become incorporated into the calcite. This is clearly not the case. The additional phosphorus does not enter the calcite and is lost but the process is unclear. The most likely explanations are as follows: As the cyanobacteria and associated algae decay, phosphorus compounds are returned immediately or via bacterial/fungal intermediates to the water. Alternatively, living cyanobacteria may be removed from the surface crust by grazing (Garcia-Pichel et al., 2004), abrasion or as hormogonia, carrying off the phosphorus with them. The latter seems unlikely to account for all of the loss since many of the taxa occur just below the surface of the crust

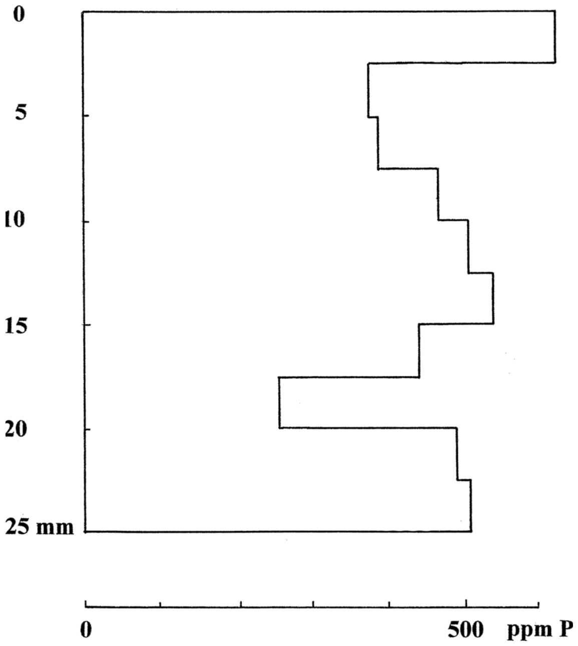

**Fig. 5** Profile of total phosphorus in carbonate crusts at site 5 (Ingleton) to a depth of 25 mm below the surface

where they are protected from abrasion. It should also be noted that some detrital organic matter of diverse origin was present in some of the crusts (e.g. leaf fragments, moss stems) that must contribute to the organic P level.

The weak but positive correlation between dissolved orthophosphate concentration in the stream water and levels in the surface crust is of interest. It is supported by studies in the Florida Everglades where periphyton phosphorus levels were found to be related to dissolved phosphorus concentrations (Gaiser et al., 2004) although further work is required as it may only be relevant to nutrient-poor streams. Had historic water chemical data been available for site 1 near Cambridge, the correlation would probably have been stronger. If such a relationship holds, it might be possible to estimate historic P levels in waters associated with much older deposits of travertine and tufa although some P-enrichment associated with diagenesis has been observed (Pentecost, 2010).

## Inhibition of crystal growth

There have been several laboratory studies of calcite crystal growth in the presence of orthophosphate (Reddy, 1977; Meyer, 1984) and they have been supported by field and theoretical investigations (Casanova & Lafont, 1985; Bono et al., 2001; Zhang et al., 2012). Relevant data are summarised in Table 4 showing that inhibition of crystal growth becomes significant in the range 50–100 µg/l orthophosphate phosphorus. These levels are considerably higher than those reported here demonstrating that current crust formation would not be influenced by them, with one possible exception, site 1. This site, the Bourn Brook and several others near Cambridge (Fig. 1 inset), all lost their *Phormidium* crusts many years ago. Recent OP analyses from these sites are shown in Fig. 1 and demonstrate values in the range 230–1200 µg/l but levels in Hobson's Brook were lower yet without encrustations. Calcite saturation quotients exceeded unity in all cases, including site 1 on the Borne Brook demonstrating supersaturation with calcite and the potential for its precipitation (Table 1). Although it is likely that high historic OP levels have led to the demise of crusts at the Cambridge sites, at least two other factors have probably exacerbated the problem. Sections of the Bourn Brook were dredged in the 1970s, and the brook was re-aligned a few years later leading to much disruption of the stream bed. In addition, water flow has been reduced since the 1940s due to high rates of abstraction for the city of Cambridge and surrounding farms. This last activity is most likely the cause for the loss of crusts in Hobson's Brook since slow flow rates have led to silt and clay deposition. Reduced flow will also limit $CO_2$ loss to the atmosphere, a process that augments crust formation (Pentecost, 2005). The phosphorus inhibition hypothesis also gains support from the unusually high levels of P in the historic crusts from the Bourn Brook indicating that even at this time, OP levels were probably high and close to becoming inhibitory.

Many thousand OP measurements are available in the databases held by the English Environment Agency and a large sample of these has been summarised by Muscutt & Withers (1996). A statistical analysis shows that over 95% of the OP levels were above the inhibitory level of 100 µg/l. Unfortunately, the great majority of the sampling sites were in the middle or lower reaches of rivers where P levels would be expected to be higher, and upper reaches, including springs were rarely sampled. Another large study of phosphorus concentrations in English river waters studied by Whitton & Neal (2011) gave a similar distribution of OP levels. It is concluded that while the majority of calcareous spring waters in regions of low population and agricultural activity will not be affected by inhibitory P levels, most lowland waters in more densely populated areas are probably no longer suitable for cyanobacterium crust formation

**Table 4** Data on the inhibitory concentrations of dissolved orthophosphate phosphorus for calcite crystal growth

| Reference | Inhibitory level of orthophosphate-*P* µg/l | Notes |
|---|---|---|
| Reddy (1977) | 30–60 | Halved crystal growth rate, laboratory study |
| Meyer (1984) | 30–100 | 50–80% reduction in crystal growth rate, laboratory study |
| Casanova & Lafont (1985) | ca. 50 | Crust inhibition observed in the field |
| Bono et al. (2001) | 100 | Field and theoretical measurements |

in England. Although phosphorus is frequently cited as the cause of the inhibition, Meyer (1984) found that a wide range of substances reported from fresh waters could be inhibitory including $Fe^{2+}$ and $Zn^{2+}$ and this also needs to be borne in mind.

For cyanobacteria to thrive in waters containing low levels of phosphorus, the cells must have a strong affinity for dissolved orthophosphate. It is conceivable that they may be able reduce the orthophosphate concentration sufficiently in their immediate vicinity to permit calcification under conditions where OP in the bulk medium would be inhibitory. Modelling the uptake of P in cyanobacterium mats is intractable without some simplifying assumptions. Application of these assumptions are likely to provide order-of-magnitude estimates that should give some indication of their influence over calcification. The problem can be simplified by considering a smooth and homogenous calcified cyanobacterium mat on a uniform carbonate substratum overlain by flowing water (Fig. 6a). The maximum rate of photosynthesis is taken at the mat surface. Given a typical water flow rate in these situations of 50 cm/s, the diffusion boundary layer will be around 25 µm thick (Dreybrodt et al., 1992). If maximum photosynthesis of the mat occurs at the surface, its thickness may be neglected and Fick's law of diffusion applied, $Q = D\,(c_1 - c_2)/t$, where $Q$ is the flux of phosphate ions through the boundary layer, $t$ the layer thickness and $c_1 - c_2$ the concentration difference between the top of the layer (i.e. the bulk concentration) and the photosynthesising layer. $D$ is the diffusion coefficient for orthophosphate, $3.6 \times 10^{-6}$ cm$^2$/s (Li & Gregory, 1974). The flux of phosphorus to the photosynthesising cells can be estimated from known rates of photosynthesis in calcified cyanobacterium mats. An upper limit in UK sites is around 2 g C/m$^2$ d (Pentecost, 2005). Carbon uptake rates can be converted to P uptake rates with a knowledge of the C:P ratio for cyanobacteria. A 'favourable' mass ratio of 28:1 was used from data in the literature (Islam & Whitton, 1992; Gibson, 1993). These data provide a flux ($Q$) of 0.9 µg P/m$^2$ s. There are now estimates of all values apart from the two P concentrations. For a typical inhibitory concentration of orthophosphate phosphorus, a value of 100 µg/l is taken (Table 4). Calculation then shows that $c_2$, the concentration at the photosynthesising surface will then be >99.9 µg/l demonstrating that the uptake of P by the mat would have no significant effect on reducing P levels in the water.

Maximum photosynthesis probably takes place a short distance below the surface of the mat as shown in Fig. 6b. Available data (Stahl, 2000) suggest a depth of about 1 mm. This additional distance increases the diffusion path, but diffusion must now occur through the calcite crust and requires an apparent diffusivity

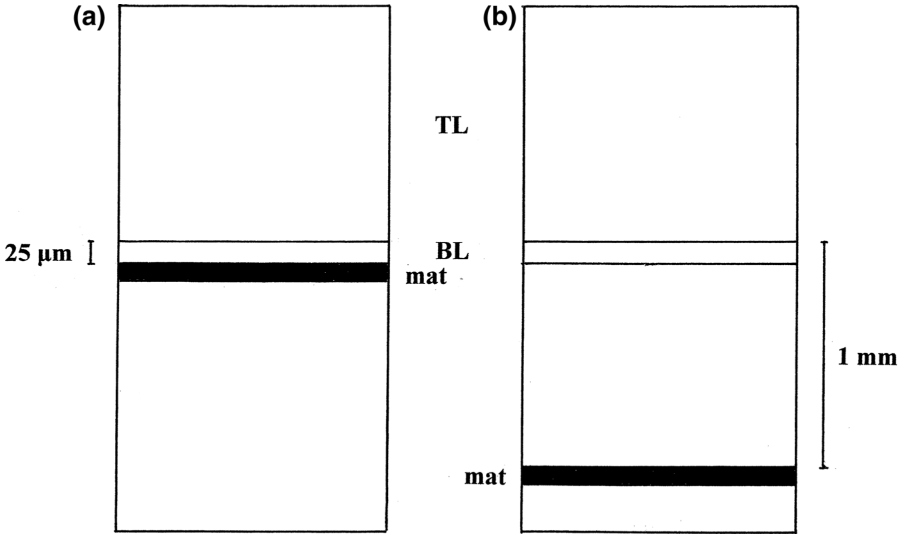

**Fig. 6** Simplifying assumptions for the transport of P into a calcified cyanobacterium mat. *TL* Turbulent layer, *BL* boundary layer **a** with maximum photosynthesis at the surface **b** with maximum photosynthesis 1 mm below the surface

factor ø in the diffusion equation. A value of 0.33 was applied based upon the porosities of carbonate crusts (Ulmann & Aller, 1982; Revsbech et al., 1998). When the above values of $Q$ and $c_1$ were inserted in the diffusion equation, $c_2$ fell to around 93 µg/l. Despite the assumptions, these calculations suggest that uptake of P by a growing cyanobacterium mat is unlikely to have a significant influence over precipitation of the associated calcite.

## Conclusions

The concentration of dissolved orthophosphate was determined at thirteen English stream crust sites and in all but one site, the levels were well below that known to inhibit the nucleation and growth of crystals of calcium carbonate as calcite.

No evidence was found for the interaction of phosphorus between the mineral and organic fractions of the crusts.

Carbonate crust formation is restricted to upland, low population density areas of England since most of the lowland waters contain inhibitory concentrations of orthophosphate.

No evidence was found to support the hypothesis that cyanobacterial uptake of phosphorus could alleviate the effects of inhibition by locally reducing the phosphorus concentration.

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