Spatial and temporal variability of biogenic isoprene emissions from a temperate estuary

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[1]   Isoprene is important for its atmospheric impacts and the ecophysiological benefits it affords to emitting organisms; however, isoprene emissions from marine systems remain vastly understudied compared to terrestrial systems. This study investigates for the first time drivers of isoprene production in a temperate estuary, and the role this production may play in enabling organisms to tolerate the inherently wide range of environmental conditions. Intertidal sediment cores as well as high and low tide water samples were collected from four sites along the Colne Estuary, UK, every six weeks over a year. Isoprene concentrations in the water were significantly higher at low than high tide, and decreased toward the mouth of the estuary; sediment production showed no spatial variability. Diel isoprene concentration increased with light availability and decreased with tidal height; nighttime production was 79% lower than daytime production. Seasonal isoprene production and water concentrations were highest for the warmest months, with production strongly correlated with light ($r^2 = 0.800$) and temperature ($r^2 = 0.752$). Intertidal microphytobenthic communities were found to be the primary source of isoprene, with tidal action acting as a concentrating factor for isoprene entering the water column. Using these data we estimated an annual production rate for this estuary of 681 μmol $m^{-2} y^{-1}$. This value falls at the upper end of other marine estimates and highlights the potentially significant role of estuaries as isoprene sources. The control of estuarine isoprene production by environmental processes identified here further suggests that such emissions may be altered by future environmental change.

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1. Introduction

[2] The volatile organic compound (VOC) isoprene (2-methyl-1,3-butadiene) is one of the most reactive and abundant hydrocarbons emitted to the atmosphere, with a concentration only marginally lower than methane [*Sharkey et al.*, 2008]. Its rapid oxidation means isoprene plays an important role in atmospheric chemistry; increasing the residence time of greenhouse gases [*Poisson et al.*, 2000] and inducing the formation of tropospheric ozone, one of the most powerful greenhouse gases. The latter is particularly evident when mono-nitrogen oxides (NOx) are abundant in the atmosphere [*Fehsenfeld et al.*, 1992; *Monson and Holland*, 2001]. Most studies have been performed on land, but interactions between isoprene and NOx from shipping have been proposed as the source of increased ozone in the southern Atlantic Ocean [*Williams et al.*, 2010].

Previous studies have also indicated an important role of isoprene in cloud formation, contributing an estimated 5–25% of total biogenic secondary organic aerosols (SOA) via atmospheric photooxidation [*Claeys et al.*, 2004], although subsequent oceanic research suggests that marine impacts on SOA are minimal [*Claeys et al.*, 2010], and more data are required to confirm this [*Shaw et al.*, 2010]. However, recent laboratory studies have suggested that isoprene could in fact significantly inhibit new aerosol formation, thus driving cloud formation in a negative feedback to climate change [*Kiendler-Scharr et al.*, 2009]. Despite this ongoing debate, isoprene clearly plays an important role in weather patterns, either providing a negative feedback to climate change through cloud formation [*Meskhidze and Nenes*, 2006], or increased warming through cloud inhibition [*Ziemann*, 2009], with major implications for future climate change, for better or worse.

[3] The majority of Earth's biogenically synthesized isoprene comes from terrestrial plants [*Sharkey and Yeh*, 2001; *Sharkey et al.*, 2008], although a handful of studies have also demonstrated significant production by marine phytoplankton [*Acuña Alvarez et al.*, 2009; *Baker et al.*, 2000; *Bonsang et al.*, 2010; *Broadgate et al.*, 1997; *Exton et al.*, 2010; *McKay et al.*, 1996; *Meskhidze and Nenes*, 2006; *Milne et al.*, 1995; *Moore et al.*, 1994; *Shaw et al.*, 2003] and macroalgae

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[*Broadgate et al.*, 2004]. The main ecophysiological benefits of isoprene biosynthesis are thought to be thermotolerance through stabilization of lipid membranes [*Siwko et al.*, 2007; *Velikova et al.*, 2006], and protection from oxidative stress [*Loreto and Velikova*, 2001; *Velikova et al.*, 2008; *Vickers et al.*, 2009]. Furthermore, recent research has demonstrated that isoprene can act as an infochemical to insects [*Laothawornkitkul et al.*, 2008; *Loivamaki et al.*, 2008], and even benefits marine heterotrophic bacteria by providing a carbon and energy source [*Acuña Alvarez et al.*, 2009]. Despite extensive research into isoprene production in terrestrial environments [*Sharkey and Yeh*, 2001; *Sharkey et al.*, 2008], relatively little is currently known regarding its production and role in marine systems. This focus on the terrestrial biome is undoubtedly due to the considerably lower estimated emission rates from marine relative to terrestrial systems. The majority of models have estimated a total global marine emission of 0.1–1.9 Tg C yr^{-1} [*Arnold et al.*, 2009; *Broadgate et al.*, 1997; *Gantt et al.*, 2009; *Luo and Yu*, 2010; *Milne et al.*, 1995; *Palmer and Shaw*, 2005], compared to ca. 400–750 Tg C yr^{-1} form terrestrial sources [*Guenther et al.*, 2006; *Müller et al.*, 2008], although more recent estimates suggest marine emissions could be as high as 11.6 Tg C yr^{-1} [*Luo and Yu*, 2010]. However, these estimates are based almost entirely on oceanic phytoplankton studies [*Baker et al.*, 2000; *Luo and Yu*, 2010; *McKay et al.*, 1996; *Milne et al.*, 1995; *Moore et al.*, 1994; *Shaw et al.*, 2003], mostly using laboratory cultures, with research into coastal sources restricted to limited work on temperate macroalgae [*Broadgate et al.*, 2004] and sediment biofilms [*Acuña Alvarez et al.*, 2009].

[4] An often overlooked factor in marine isoprene emission estimates is seasonality, with limited research demonstrating significant seasonal and diel variation in open ocean samples [*Broadgate et al.*, 1997; *Liakakou et al.*, 2007], and diel variation in macroalgal samples [*Broadgate et al.*, 2004]. Consequently, if isoprene emissions from marine systems are to be fully understood, and more accurately estimated in future, we must better understand the sources and sinks of isoprene in coastal systems, while taking into account its temporal variability and how environmental factors influence emission rates.

[5] Estuaries are highly dynamic systems that experience extreme spatial and temporal heterogeneity of factors including salinity, temperature, circulation, tidal mixing and nutrients [*Kocum et al.*, 2002; *Lawrence and Harding*, 1994]. This susceptibility to change provides a unique model system to address key questions on seasonal, diel, spatial and tidal driven variability in biogenic isoprene, as well as the impacts of corresponding factors such as temperature, light, salinity and chlorophyll *a*. In addition, although estuaries contribute only 2.5% of total marine and 1.1% of total global primary productivity, they are a key component of coastal primary productivity and yield some of the highest productivity rates per unit area for marine biomes [*Geider et al.*, 2001].

[6] Given the well established importance of temperate estuaries in both coastal productivity [*Geider et al.*, 2001], environmental variability [*Kocum et al.*, 2002], and isoprene emissions [*Acuña Alvarez et al.*, 2009], we aimed to further explore isoprene variability, on spatial and temporal scales. Based on the role of isoprene in stress protection, we hypothesized that its production will be high under fluctuating

environmental conditions experienced by estuarine communities. We used the Colne Estuary, UK, as a model system to (1) determine how the isoprene budget varies spatially along the environmental gradient of an estuary, (2) examine temporal patterns, both diel and seasonal, in the isoprene budget of an estuary, in response to biotic and abiotic factors, and (3) identify the main sources of isoprene emissions among the phototrophic community.

2. Methods

2.1. Site Description

[7] The Colne estuary is a shallow, well-mixed estuary situated in Essex, United Kingdom [*Kocum et al.*, 2002; *Ogilvie et al.*, 1997]. The estuary is mesotidal (3.5 to 4 m tidal range) [*Thornton et al.*, 2007], about 10 km long, and highly branched (total shoreline ca. 90 km) [*Kocum et al.*, 2002], with a catchment of 500 km^2 [*Dong et al.*, 2000], of which ca. 93% is agricultural land [*Environment Agency*, 2008]. Four sites were chosen to represent the full salinity gradient of the estuary (Figure 1). Site 1 was the most landward site, situated in a small-scale industrial and commercial district of a large town, and exhibited a salinity range from fully freshwater at low tide to 10 at high tide. Site 2 was situated near a village and had a salinity range of 3–31, while Site 3 was surrounded by agricultural and wetland areas and exhibited a salinity range of 9–33. Site 4 was the most seaward site, situated near a small town and harbor, with salinity ranging from 28–33.

2.2. Seasonal Sampling

[8] To assess seasonal variation in isoprene emissions from the Colne Estuary, the following were examined at each site: (1) isoprene concentrations in estuary water samples at both high and low tide, and (2) isoprene production rates in intertidal mudflat microphytobenthos communities. Preliminary incubation experiments showed that isoprene production rates in the water column were undetectable, and so only concentrations were measured. Water samples were collected in triplicate from the center of the estuary in 1 L acid-washed glass containers lowered to 0.3 m below the surface. For sediment analysis, triplicate cores (10 cm diameter, 8 cm depth) were taken at low tide from the exposed intertidal zone at a mid-tide elevation. Sampling was repeated eight times, every 6–8 weeks, from March 2009 to April 2010. The sampling procedure was standardized by sampling between 09:00 and 11:00 h, as close to low/high tide as logistically possible, and samples were processed immediately on return to the laboratory (within 2 h of sampling). The sampling date was selected such that low tide sampling occurred between 0.7–1.2 m tidal height, and high tide sampling occurred between 3.5–4 m.

[9] Air temperature was measured during sediment sampling, and surface water temperature was measured during water sampling, at each site using a mercury thermometer accurate to 0.5°C. Light intensity was measured at all sampling times and locations, using a Li-Cor LI-250A handheld light meter with flat sensor. A handheld refractometer was used to measure water salinity on the practical salinity scale. From each water sample, 50 ml was filtered through GF/F glass fiber filters, 0.7 μm pore size (MF300, Fisher Scientific, Massachusetts, USA), and the filters flash frozen in liquid nitrogen and stored at −80°C for chlorophyll analysis at a later
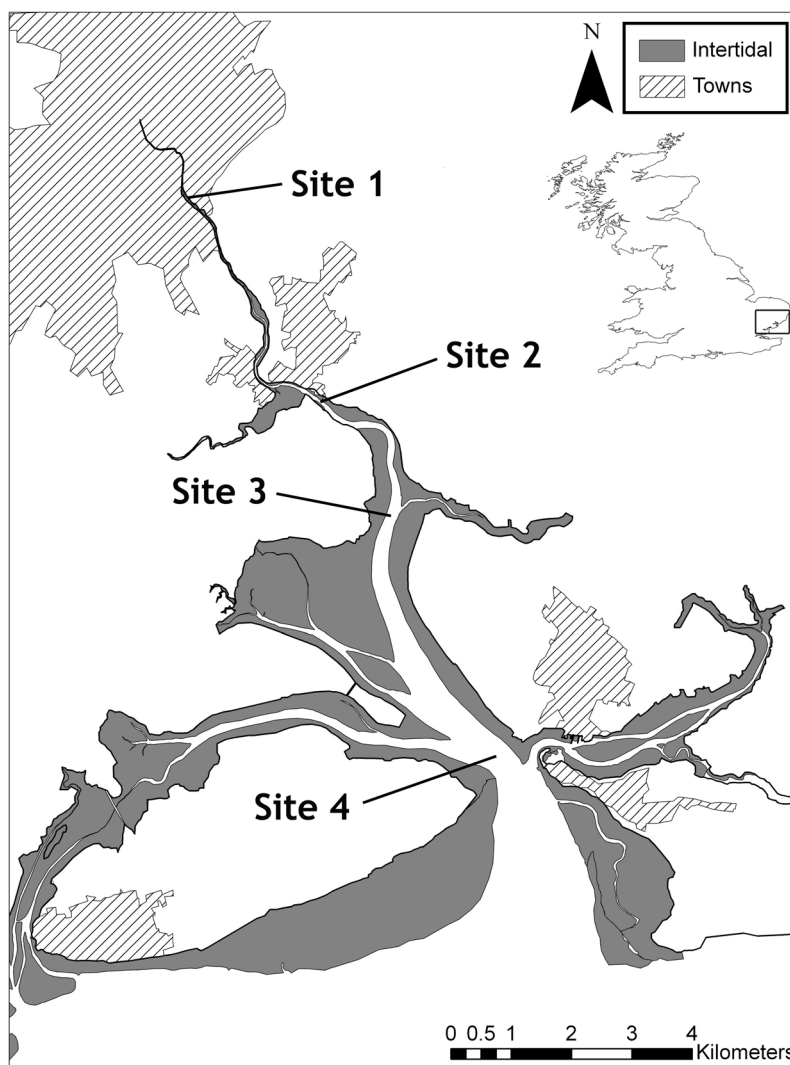
Figure 1. Map of the Colne Estuary, UK, showing the four sample collection sites: Site 1 (Hythe, 51°52′ 48.58″N 0°55′41.32″E); Site 2 (Wivenhoe, 51°51′08.23″N 0°57′46.27″E); Site 3 (Alresford, 51°50′19.38″N 0°58′54.77″E); Site 4 (Brightlingsea, 51°48′19.67″N 1°00′06.53″E). Major settlements are shown in cross-hatch, and intertidal zones are shaded in gray.

date. Sediment sub-cores were also taken, freeze-dried and stored at −80°C for similar analysis.

2.3. Diel Sampling

[10] Site 1 was selected for two-hourly sampling over a 24 h period from 8–9 July 2010. Water samples were collected in triplicate from the center of the estuary every 2 h, and returned to the laboratory for analysis as above. Upon return to the laboratory, samples were immediately analyzed in sampling sequence which resulted in an average storage time of 2 h for each sample. Measurements of light intensity, water temperature and tidal height were taken at each time point. All times are given in GMT.

2.4. Isoprene Measurements

[11] From each water sample, 60 ml was filtered through a GF/F glass fiber filter, 0.7 μm pore size (MF300, Fisher Scientific, Massachusetts, USA), into a glass purge vessel

[Exton et al., 2010]. The vessel was then purged for 20 min using high purity nitrogen gas (BOC Gases, UK) at a flow rate of 80 ml min^{-1}, and the released gases passed through a stainless steel cryo-trap held at −160°C using a liquid nitrogen boiler [Vogt et al., 2008]. For sediment samples, four sub-cores (2 cm diameter, 2 cm depth) were taken from each sample core, and incubated together in gas-tight glass purge vessels, at temperature and light levels closely mimicking ambient conditions at the time of sampling, for between 2 and 4 h. The vessels were then purged for 30 min and stored in a cryo-trap (as for water samples). Isoprene was quantified using a gas chromatograph (GC-2010; Shimadzu, Milton Keynes, UK) with flame ionization detection (GC-FID), and fitted with a GC-Al/KCl column of 50 m length, with a 0.53 mm internal diameter and ultra-high-purity (UHP) helium carrier gas. The FID was set to 250°C, with UHP hydrogen (40 ml min^{-1}) and zero-air (400 ml min^{-1}) as flame gases, and UHP nitrogen as a make-up gas
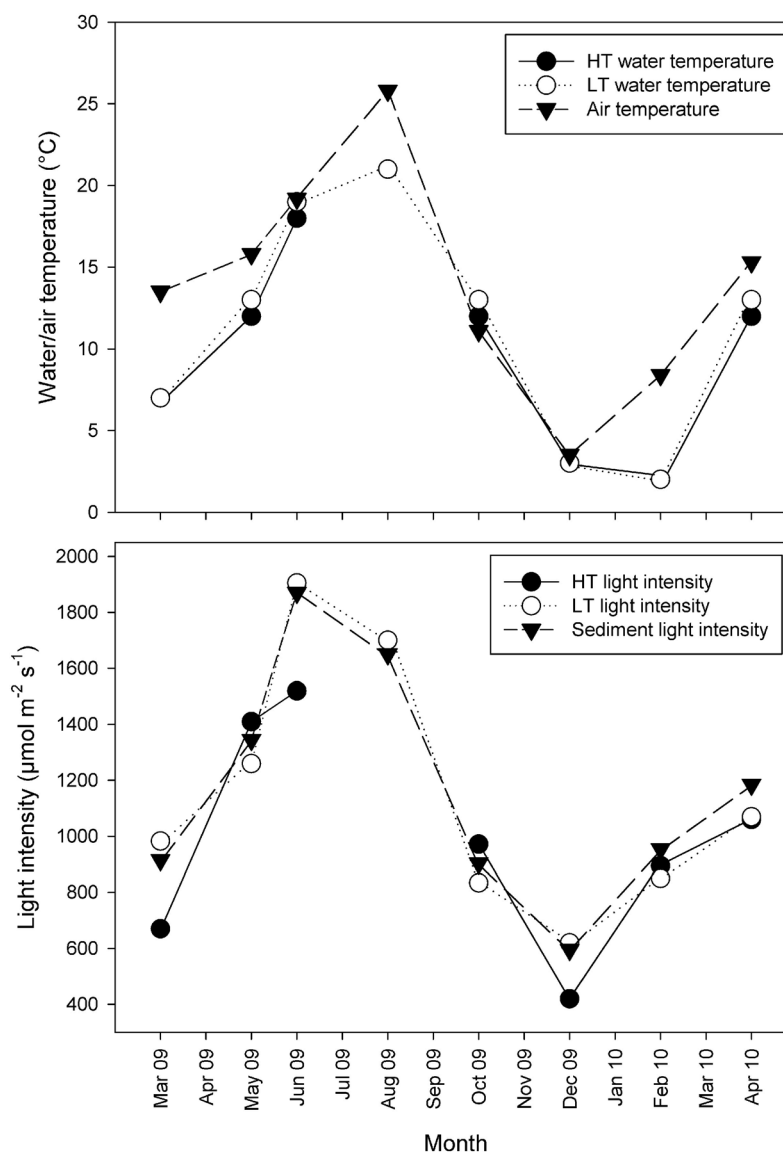
Figure 2. Seasonal variation (March 2009 to April 2010) in surface water temperature at low tide (LT) and high tide (HT), air temperature above the sediment, and light intensity. Data from Sites 1 to 4 were pooled for clarity (n = 12). Standard error was smaller than the symbol size.

(30 ml min^{-1}). The GC temperature profile began at 80°C for 3 min, before increasing at 40°C min^{-1} to 200°C and held for 12 min. Calibration was performed using a 100 ppmv isoprene standard gas in helium (Scientific and Technical Gases Ltd., UK). The isoprene recovery efficiency in purged samples was shown to be >95%.

[12] An annual isoprene production rate for the Colne Estuary was estimated as follows using data from benthic incubation experiments:

$$\sum \left\{ \left[\left(I_{prod} \times t_{day} \right) + \left(I_{prod} \times \%_{night} \times t_{night} \right) \right] \times (365 \div 8) \right\} \quad (1)$$

Where I_{prod} = mean hourly isoprene production rate for all four sites at each sampling time point (μmol m^{-2} h^{-1}), t_{day} and t_{night} = day (light) and night (dark) length, respectively, for the Colne Estuary at that time of year (hours per day),

$\%_{night}$ = hourly dark (night) isoprene production as a percentage of daylight production (dimensionless). The conversion factors 365 and 8 account for the period of the year each of the eight sampling sessions represents (days).

2.5. Chlorophyll Analysis

[13] For chlorophyll extraction, each filter was ground in 5 ml of 100% methanol and kept in darkness at −20°C overnight. The same extraction method was used for sediment, except 10 ml of methanol was used [*Bellinger et al.*, 2005; *Thornton et al.*, 2002]. Samples were then centrifuged for 5 min at 1800 *g* (Mistral 2000 Centrifuge; MSE, London, UK). Supernatant absorption between 600 and 760 nm was read using a spectrophotometer (U-3000; Hitachi High Technologies, Wokingham, UK). Chlorophyll *a*, *b* and *c* concentrations were quantified using published equations for

Table 1. Results From a Kruskal-Wallis One-Way Analysis of Variance Showing Sample Number (n), Degrees of Freedom (df), Statistical Value (H), and Probability Value (P)[a]

Variables Tested	n	df	H	P
Spatial Variation in Isoprene Concentration				
HT isoprene between sample sites	84	3	36.19	<0.001
LT isoprene between sample sites	96	3	14.63	<0.05
LT isoprene > HT isoprene	142	1	5.93	<0.05
Diel Variation in Isoprene Concentration				
Diel isoprene variation	36	11	31.31	<0.05
04:00 peak > 16:00 peak	6	1	3.86	<0.05
Seasonal Variation in Isoprene Concentration				
HT isoprene between sample months	84	6	25.88	<0.001
LT isoprene between sample months	96	7	40.76	<0.001
Seasonal Variation in Isoprene Production				
Benthic isoprene between sample months	96	7	69.01	<0.001
Seasonal Variation in Chlorophyll Concentration				
HT chlorophyll *a* between sample months	84	7	59.59	<0.001
LT chlorophyll *a* between sample months	96	7	46.09	<0.001
Benthic chlorophyll *a* between sample months	96	7	45.42	<0.001
Benthic chlorophyll *b* between sample months	96	7	57.16	<0.001
Benthic chlorophyll *c* between sample months	96	7	45.83	<0.001

[a]Note that a higher value for H represents increased significance. HT refers to high tide water sampling, LT refers to low tide water sampling, benthic refers to intertidal sediment core sampling of microphytobenthos. Only significant results are shown. Two time points (04:00 and 16:00 h) are specifically compared for diel sampling as they represent the day- and nighttime maxima in isoprene water concentrations.

natural phytoplankton communities [*Ritchie*, 2008], and used to derive both algal biomass (chlorophyll *a*) and broad changes in community assemblage via ratios of the chlorophyll species. Chlorophyll concentrations were not corrected for potential contribution of phaeopigments, via subsequent re-analysis post acidification of samples, given the potential for high inaccuracy using this technique on estuarine sediment samples [*Brotas et al.*, 2007].

3. Results

3.1. Physico-chemical Characteristics

[14] The only noticeable variation in physico-chemical parameters between sites was for salinity (see section 2.1, Site Description). Spatial variation in water and air temperatures were typically <1°C at any sampling time and, together with light intensity, differences were statistically insignificant. As expected, seasonal air and water temperatures, as well as light intensity, peaked during the summer months, although light intensity was highest in June whereas temperature peaked in August (Figure 2). Throughout the study, water temperature varied by ca. 20°C, and air temperature by ca. 25°C. Sediment light intensity at low tide at the time of sampling ranged from 570–1870 μmol m^{-2} s^{-1} over the year.

3.2. Spatial Variation in Isoprene Concentration (Water Samples) and Production (Sediment Samples)

[15] Averaged across the year, isoprene concentrations in water samples showed statistically significant variation between sites at both high tide (HT) and low tide (LT) (Table 1), decreasing in a seaward direction toward Site 4 (Figure 3). Highest values were found at the head of the estuary, Site 1 (HT: 164.9 pmol L^{-1}; LT: 194.3 pmol L^{-1}), and lowest values at the seaward end, Site 4 (HT: 27.5 pmol L^{-1}; LT: 56.0 pmol L^{-1}). Additionally, isoprene concentrations in the water were significantly higher at LT than at HT (Table 1 and Figure 3).
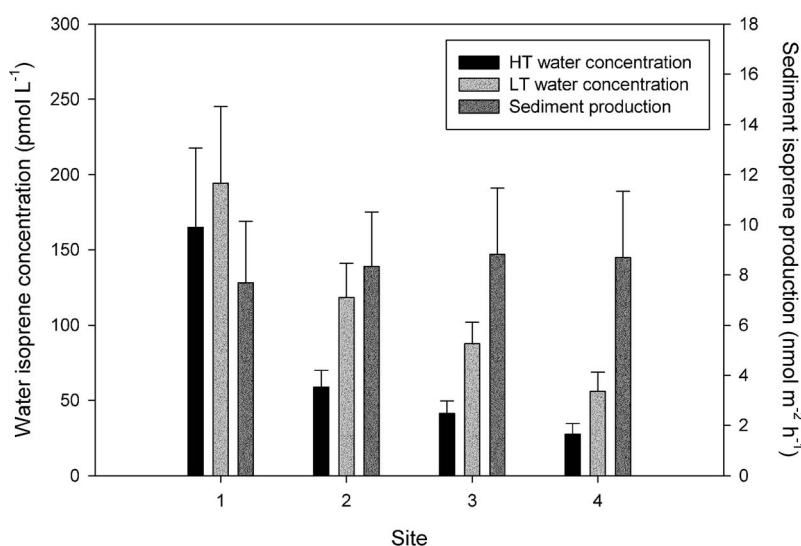

Figure 3. Spatial variation in isoprene concentration (water) and production (sediments) for Sites 1 to 4 along the Colne Estuary, determined as the mean ± standard error (SE) for the entire sampling period (March 2009–April 2010, n = 23). Data are for high-tide (HT) and low-tide (LT) water concentrations and intertidal benthic sediment production.

Table 2. Pearson's Correlation Data Showing Sample Number (n), Correlation Coefficient (r^2) and Probability Value (P)[a]

Variables Tested	n	r^2	P
Diel Variation in Isoprene Concentration			
Isoprene against tidal height	36	−0.747	<0.001
Seasonal Variation in Isoprene Concentration			
HT isoprene against HT chlorophyll *a*	84	0.332	<0.05
LT isoprene against LT chlorophyll *a*	96	0.344	<0.05
Seasonal Variation in Isoprene Production			
Benthic isoprene against benthic chlorophyll *a*	96	0.316	<0.05
Benthic isoprene against air temperature	96	0.752	<0.001
Benthic isoprene against light intensity	96	0.800	<0.001
Benthic isoprene against chlorophyll *c*	96	−0.458	<0.001

[a]HT refers to high tide water sampling, LT refers to low tide water sampling, benthic refers to intertidal sediment core sampling of microphytobenthos. Only significant results are shown.

[16] Conversely, isoprene production from intertidal sediment samples showed no variation along the estuary, with mean production rates of 8.38 ± 1.18 nmol m^{-2} h^{-1} across all sites (Figure 3).

3.3. Diel Variation in Water Column Isoprene Concentration

[17] The concentration of isoprene in water over the 24-h period showed a strong negative correlation with tidal height (Table 2 and Figure 4). Water isoprene concentrations peaked twice, reaching 408.0 pmol L^{-1} at 16:00 and 244.3 pmol L^{-1} at 04:00, both coinciding with the lowest tidal heights (1.3 m and 1.1 m respectively). Conversely, the three minimum isoprene concentrations (127.0 pmol L^{-1} at 10:00; 102.2 pmol L^{-1} at 22:00; 102.1 pmol L^{-1} at 08:00) coincided with the greatest tidal heights (3.6 m, 4.0 m and 3.4 m respectively). Isoprene concentration at the 16:00 peak was higher than at 04:00 (Table 1), and corresponded with an increase of both water temperature and light intensity (Figure 4). Water temperature varied from 25°C at 18:00 to
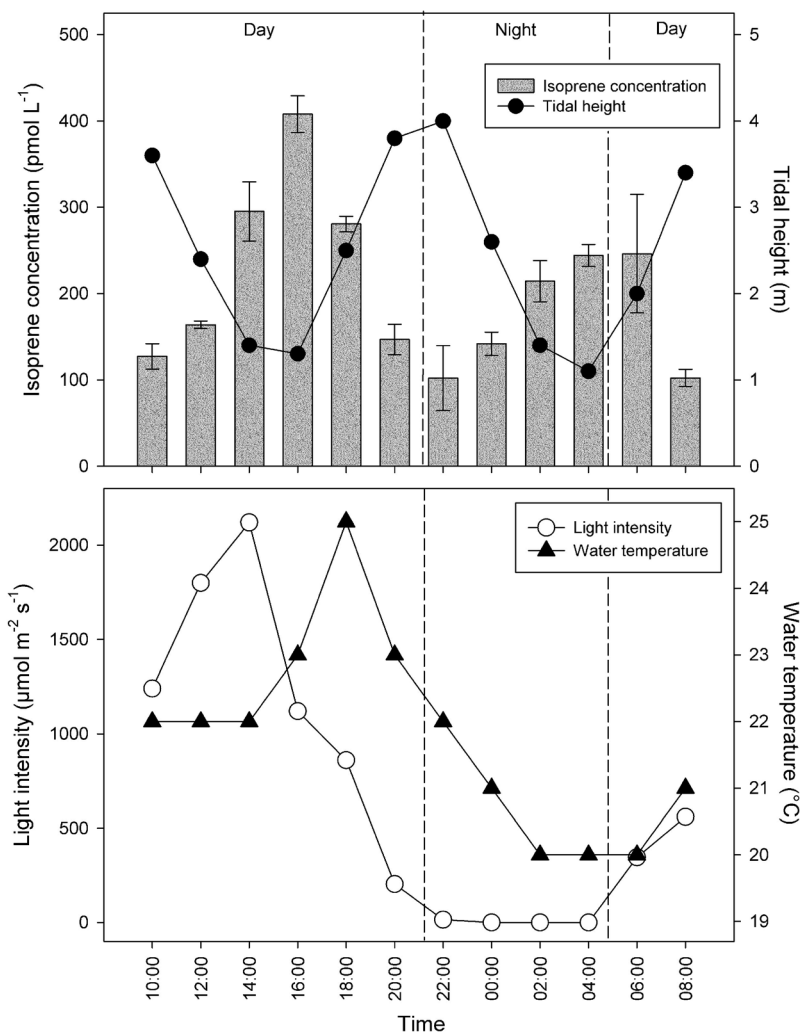

Figure 4. Diel variation of isoprene water concentration at Site 1 (head of the Colne estuary) on 8–9 July 2010. (top) Tidal height and isoprene (mean ± SE, n = 3) and (bottom) light intensity and surface water temperature. See Table 2 for additional statistical information.
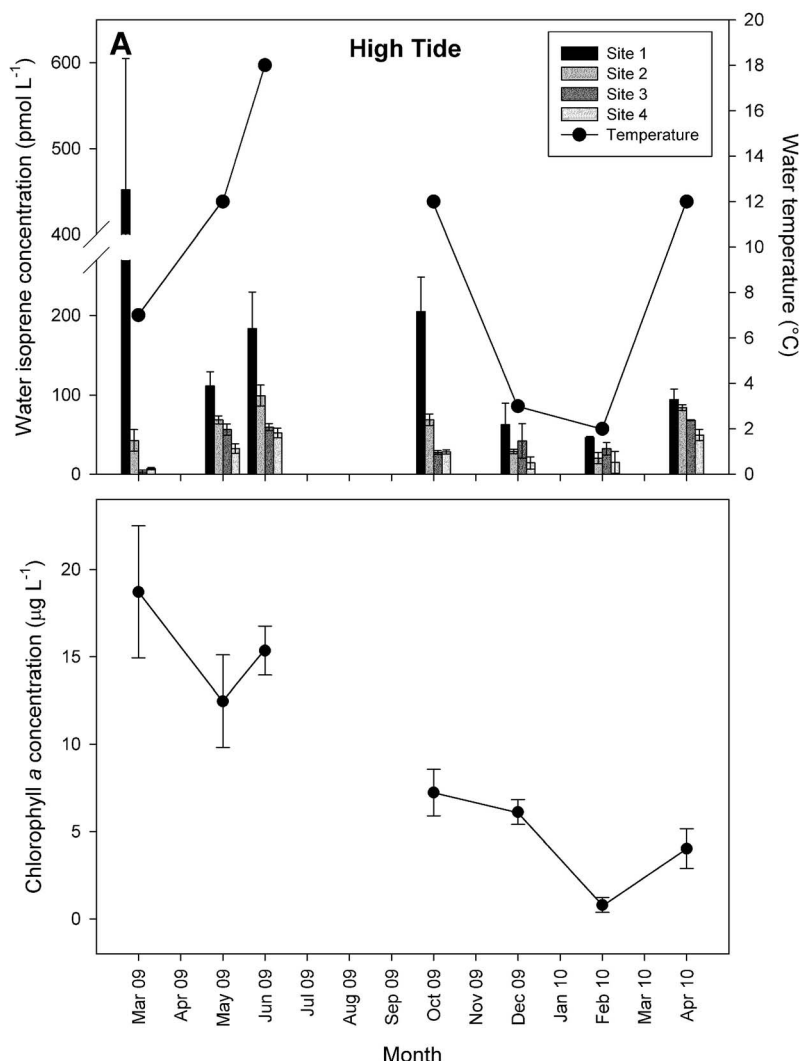
Figure 5. Seasonal variation in isoprene and chlorophyll a at four sites along the Colne Estuary for (a) high-tide water column isoprene and chlorophyll a concentration and water temperature, (b) low-tide water column isoprene and chlorophyll a concentration and water temperature, and (c) intertidal benthic isoprene production, chlorophyll a concentration, and air temperature. Isoprene concentrations and production rates at each site and month show the mean $\pm$SE (n = 3), while temperature and chlorophyll a concentrations show the mean across all sites $\pm$SE (n = 12). Please note that high-tide data are unavailable for August 2009 due to logistical difficulties.

$20°$C between 02:00 and 06:00, while light intensity ranged from 2120 μmol m^{-2} s^{-1} at 14:00 to zero between 22:00 and 04:00; but no correlation was found over the 24 h period between isoprene concentration and either temperature or light. However, stepwise multiple regression analysis revealed that when temperature was added to the initial model of inverse tidal height (R^2_{adj} = 50.18), there was an increase in the model fit of 24% (to R^2_{adj} = 74.25), while the addition of light did not increase the model fit. This indicates that temperature is a key driver of diel variability in isoprene concentration, along with tidal height.

[18] In order to examine dark-driven isoprene production, intertidal sediment samples from Site 1 were incubated as described in 2.4, but with light and dark treatments. Dark-

incubated samples produced 78.8% less isoprene than those incubated in the light, although detectable production rates were still observed in the dark (1.3 $\pm$ 0.2 nmol m^{-2} h^{-1}).

3.4. Seasonal Variation in Isoprene Concentration (Water Samples) and Production (Sediment Samples)

[19] HT water isoprene concentrations at all sites followed a similar seasonal pattern to water temperature and light intensity, with high values during the summer and low values during the winter (Figure 5a), although no significant correlations were found. However, HT water isoprene concentrations were correlated with HT water chlorophyll a concentrations across the year (Table 2). Minimum HT isoprene concentrations occurred at Sites 1 and 2 in February
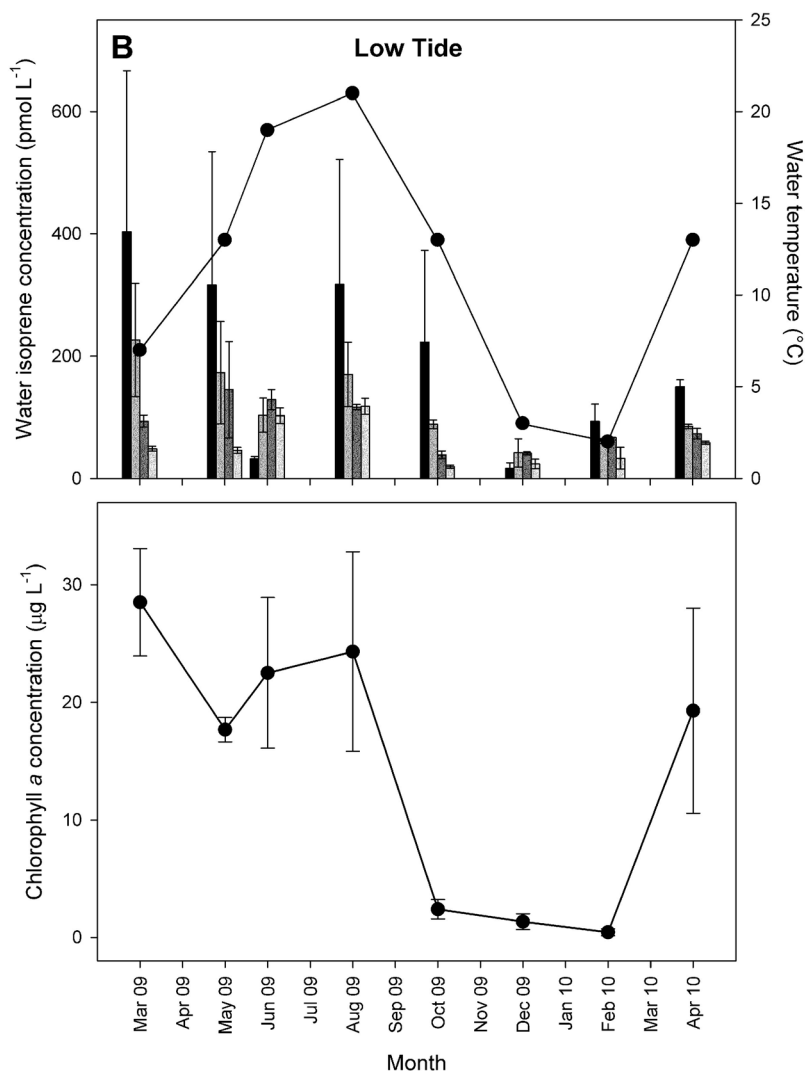
Figure 5. (continued)

(ca. 20–45 pmol L^{-1}) and Sites 3 and 4 in March (ca. 1–3 pmol L^{-1}). Despite August values for HT being unavailable due to logistical problems, isoprene concentrations increased at all sites during the warmer months, with maximum values for Sites 2 and 4 in June (ca. 50–100 pmol L^{-1}) and Site 3 in April (68.1 pmol L^{-1}). Although Site 1 showed a pronounced peak in March (451.8 pmol L^{-1}), the next highest values were in June (183.5 pmol L^{-1}) and October (205.0 pmol L^{-1}).

[20] LT samples followed a less defined seasonal pattern in isoprene water concentration, but were also correlated with chlorophyll a concentrations (Table 2), but not with temperature or light. Sites 3 and 4 followed a similar pattern to HT concentrations, with winter lows (Figure 5b). Maximum concentrations were found at Site 3 (144.9 pmol L^{-1}) in May and Site 4 (117.9 pmol L^{-1}) in August, while minimum concentrations were found at both sites in October (ca. 20–40 pmol L^{-1}). Sites 1 and 2 showed consistently low concentrations during the winter, with minimum concentrations for both sites in December (ca. 15–40 pmol L^{-1}), but high temperature maxima were less distinct. Both sites had

maximum isoprene concentrations in March (ca. 225–405 pmol L^{-1}), but decreased in June, particularly Site 1 which decreased to a level similar to winter values (32.1 pmol L^{-1}).

[21] Sediment isoprene production rates also showed summer maxima (Figure 5c), strongly correlated with air temperature and light (Table 2). As with both HT and LT samples, isoprene sediment production rates were correlated with chlorophyll a (Figure 6 and Table 2), although this result appears to be strongly influenced by the consistently low values of both isoprene production and chlorophyll a concentration from winter sampling sessions (October, December and February). Isoprene sediment production peaked during August at all sites (ca. 20–25 nmol m^{-2} h^{-1}) except Site 4, which peaked in May (22.96 nmol m^{-2} h^{-1}). Minimal values for each site were recorded over the winter period between October and February (ca. 0.5–2 nmol m^{-2} h^{-1}). Using these data with equation (1), annual isoprene production rate per unit area of intertidal sediment across the four sampling sites was calculated to be 681 μmol m^{-2} y^{-1}.
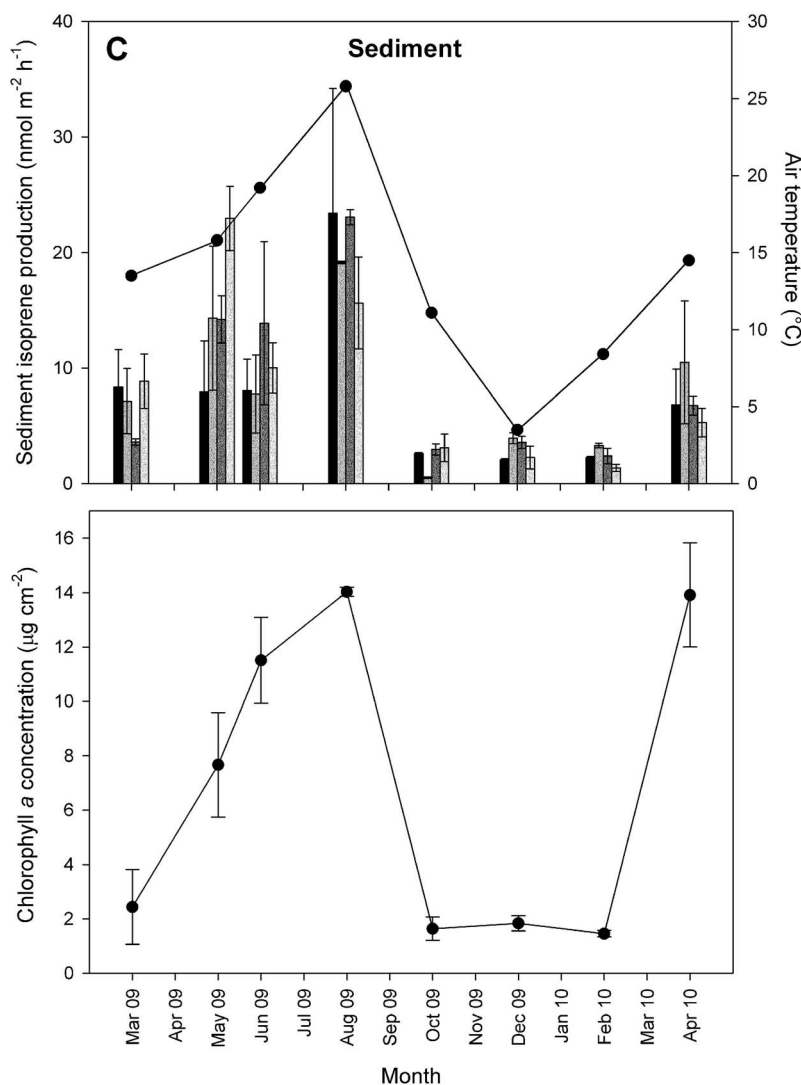
Figure 5. (continued)

3.5. Photosynthetic Biomass and Community Structure

[22] Chlorophyll *a* concentrations showed no variation between sites, for either water or sediment samples, or between tides, but did show statistically significant variation between months (Table 1). Water concentrations of chlorophyll *a* peaked in March, and again in August, while sediment concentrations were low in March and peaked in August (Figure 5). Concentrations in both water and sediment samples decreased during the autumn and winter months.

[23] In order to broadly assess changes in microphytobenthic community assemblage, ratios of chlorophyll *b:a* and *c:a* were calculated, where the increases of chlorophyll *b* and *c* relative to those of chlorophyll *a* are indicative of a predominance of euglenoid and diatom genera, respectively [*Jeffrey et al.*, 1996]. These both varied between months (Table 1), but not between sampling sites. Chlorophyll *b:a* peaked from March to May at all sites, with maxima ranging from 0.28–0.95, and values decreasing for the rest of the year (Figure 7). Chlorophyll *c:a* followed an inverse pattern, with minimal values of ca. 0.00–0.25 between April to June and

higher values of ca. 0.30–0.52 during the rest of the year. Chlorophyll *c:a* in sediment samples was negatively correlated with sediment production of isoprene (Table 2), but no correlation between isoprene and the proportion of chlorophyll *b* was found.

4. Discussion

[24] The isoprene water concentration values of this study (0.7–451.8 pmol L^{-1}) are noticeably higher than open ocean concentrations, but fit previously measured coastal ranges. In the nearby North Sea isoprene ranges from 0.7–90 pmol L^{-1} [*Broadgate et al.*, 1997], and similar studies around the globe provide values of between 9.8 and 50.8 pmol L^{-1} in the Florida Straits [*Milne et al.*, 1995], 3.6 and 98 pmol L^{-1} in the Pacific [*Bonsang et al.*, 1992] and 5 and 55 pmol L^{-1} in the Eastern Atlantic [*Baker et al.*, 2000]. The values here were similar to those found in temperate intertidal rock pools, where isoprene concentrations are shown to be 24.9–865.0 pmol L^{-1} compared to only 10.0–20.8 pmol L^{-1} in nearby open ocean samples [*Broadgate et al.*,
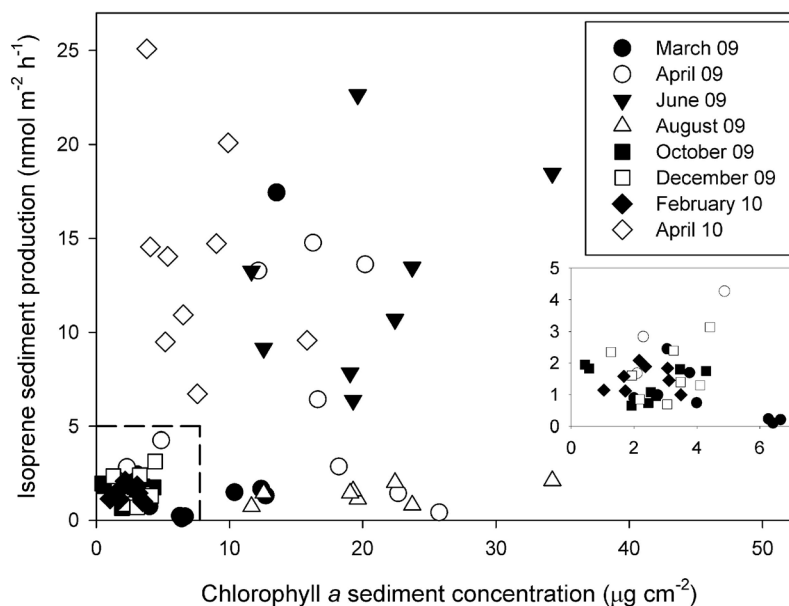
Figure 6. The relationship between sediment chlorophyll *a* concentrations and isoprene production rates in intertidal sediment samples taken from the Colne Estuary between March 2009 and April 2010 ($r^2 = 0.344$, $P < 0.05$, $n = 96$). Data from each sampling period are displayed separately.

2004]. Although slightly higher than concentrations here, it should be noted that the water volume – and thus the potential for dilution of isoprene – in rock pools sampled by *Broadgate et al.* [2004] was much lower than in the Colne Estuary. Similarly, sediment microphytobenthic isoprene production rates are similar to previous measurements taken along the Colne Estuary [*Acuña Alvarez et al.*, 2009].

[25] A number of factors point toward the high contribution of intertidal microphytobenthic communities to the total isoprene budget of the estuary. No isoprene production was detected in incubated water samples, while sediment incubation experiments provided high levels of isoprene production with lower, but still relatively high, values during periods with low temperature and light (Figure 5c). The negligible water column production measured is unlikely to be responsible for the relatively high water concentrations, due to the high volatility and reactivity of isoprene making its lifetime short (0.6–2 h) [*Liakakou et al.*, 2007]. This agrees with previous work on coastal systems, where isoprene concentrations in rockpool water samples containing macroalgae are up to two orders of magnitude higher than control samples [*Broadgate et al.*, 2004]. In the same way that macroalgae are concluded to be important sources of isoprene in rocky shore systems, this study suggests that intertidal benthic microalgae are similarly important sources of isoprene in temperate estuaries and should be considered the major contributor to isoprene water concentrations. It is also important to bear in mind the potentially considerable role of microbial isoprene consumption demonstrated in samples from the Colne Estuary [*Acuña Alvarez et al.*, 2009], suggesting that gross production rates are likely to be much higher than the net production rates reported here.

[26] The spatial variation in isoprene concentration found along the estuary is consistent with the idea of benthic microalgae being the main source of isoprene. For example, despite no significant variation in isoprene production from

the sediments, isoprene concentrations in the water column decreased with high tide and in a seaward direction (Figure 3). The seaward mouth of the estuary is characterized by a larger ratio of water volume to sediment surface area than at the head, resulting in a higher dilution potential for isoprene produced by microphytobenthic communities. The lower isoprene concentrations measured at high compared to low tide (observed on a diel and seasonal scale; Figures 4 and 5) can similarly be explained by a higher ratio of water volume to sediment surface area at high tides, and thus dilution of the isoprene from the microphytobenthos. Laboratory incubations of benthic *Cylindrotheca* sp. cultures isolated from the Colne Estuary have also shown significant production of isoprene ($0.11 \pm 0.00 \ \mu$mol (g chl *a*)$^{-1}$ h^{-1}) [*Exton*, 2012], further supporting the argument that benthic algal communities are the major source of isoprene in temperate estuaries.

[27] A notable finding from the diel analysis of water column isoprene concentrations was the presence of an isoprene peak during the night (Figure 4). Although some open ocean data suggest a single isoprene peak during daylight hours in response to temperature and light maxima [*Liakakou et al.*, 2007], our study agrees with previous work where isoprene is present during the night (2.7 pmol L^{-1}), but significantly higher during the day (5.6 pmol L^{-1}) [*Matsunaga et al.*, 2002]. More recent modeling work highlights the underestimation of nighttime isoprene emissions [*Gantt et al.*, 2009]. In the Colne, this finding could demonstrate continued isoprene production under dark conditions, supported by observed production in sediment samples incubated in the dark. Isoprene production in the dark is demonstrated by cyanobacterial cultures [*Shaw et al.*, 2003] and the temperate macroalga *Ulva intestinalis* [*Broadgate et al.*, 2004], both of which show similar decreases in production compared to light incubations as found here for sediment samples. When photosynthesis is inhibited, isoprene can be formed via the
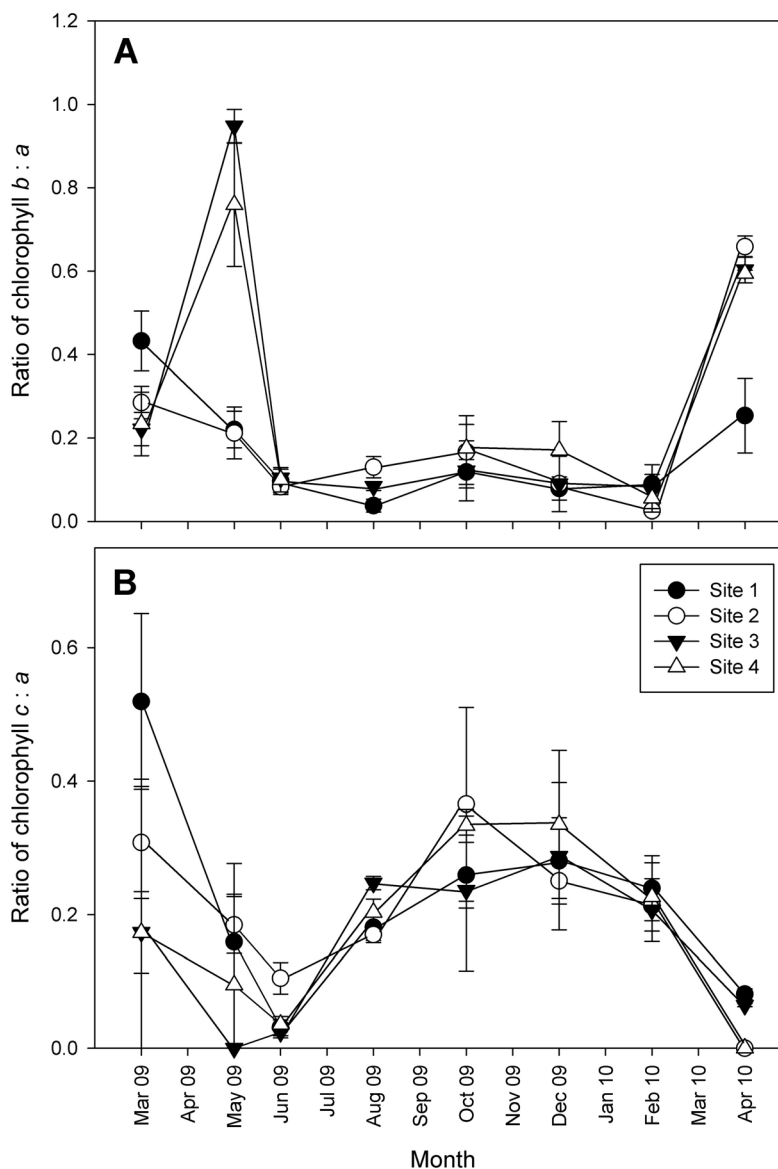
Figure 7. Ratios of (a) chlorophyll *b*:*a* and (b) chlorophyll *c*:*a* concentrations (μg cm^{-2}) in intertidal sediment samples taken from the Colne Estuary between March 2009 and April 2010. Shown are mean values ±SE (n = 3).

breakdown of stored carbohydrates [*Lerdau et al.*, 1997], while some production could be attributed to microbial communities, as found in terrestrial bacteria [*Fall and Copley*, 2000; *Kuzma et al.*, 1995]. These could help explain the isoprene concentration measured at Site 1 through the night, while low tidal height keeps the isoprene at high concentrations, illustrated by the nighttime peak in isoprene at low tide. Similarly, the increased daytime concentrations of isoprene demonstrate the influence of environmental factors, although multiple regressions analysis suggests that only temperature has a significant role alongside tidal height in driving diel variations in isoprene concentration in the Colne estuary.

[28] Benthic isoprene production rates had the most clearly defined seasonal pattern (Figure 5), with a significant increase during the spring and summer months, further supporting the idea that the microphytobenthos is the primary source of isoprene in the estuary. High- and low-tide water concentrations, although following broadly similar patterns across the year, are subjected to additional factors which could lessen the clarity of any seasonal pattern. For example, isoprene concentrations in water samples from Site 1 were unexpectedly high in March 2009, and represented the maximum concentrations for the entire year. However, benthic production rates did not match the high concentrations, and water column production rates remained undetectable. Taken together, this suggests an external input of isoprene into the estuary at this time point, and the lack of a similar peak in spring 2010 further supports this notion. Potential external sources of isoprene to the estuary were not explored, but could have been through chemical pollution from local industry, although we would not wish to speculate on specific potential sources.

[29] Relatively weak correlations suggest that variation in isoprene throughout the year can be explained partly by changes in biomass (using chlorophyll *a* as a surrogate for algal biomass) and algal community composition (characterized by the relative proportions of chlorophyll *b* and *c*) (Figures 6 and 7). The assemblage of microphytobenthic communities appears to have only a minor effect on temporal isoprene emission patterns. Changes in chlorophyll *c*, a pigment broadly associated with the presence of diatoms [*Jeffrey et al.*, 1996], appear to explain some of the temporal isoprene variability due to the negative correlation between the two, while the lack of association between isoprene and chlorophyll *b* indicates that changes in the euglenoid population have no influence. Overall, this supports the findings of *Broadgate et al.* [1997] who conclude, albeit tentatively and in contrast to similar studies for dimethyl sulfide [*Liss et al.*, 1994], that species composition has limited effect on isoprene emissions. It is important to point out, however, that laboratory studies highlight the high inter-specific variation in isoprene production rates for cultured microalgae [*Acuña Alvarez et al.*, 2009; *Colomb et al.*, 2008; *Gantt et al.*, 2009; *Milne et al.*, 1995; *Moore et al.*, 1994; *Shaw et al.*, 2003, 2010], suggesting that further taxon-specific pigment data may reveal the impact of selected taxonomic groups on isoprene variation in the Colne estuary.

[30] Correlations between chlorophyll *a* and isoprene were in contrast to previous open ocean research from the nearby North Sea, where isoprene is more strongly associated with changes in chlorophyll *a* ($r^2 = 0.54$–0.87) [*Broadgate et al.*, 1997], although similar studies elsewhere find less effect of chlorophyll *a* on isoprene [*Baker et al.*, 2000]. Despite the relatively low correlations found in this study (Table 2), biomass did appear to contribute to variability in estuarine isoprene, although it also suggests that additional driving factors are at play. This agrees with the proposed existence of two distinct regimes for marine isoprene production: biomass-related, and stress-driven [*Gantt et al.*, 2009].

[31] Coastal marine systems, particularly temperate estuaries, exhibit a high level of fine-scale environmental variability compared to the open ocean, meaning the role of isoprene in the acclimation of algae to changes in environmental conditions could be much greater in these environments. This is supported by the strong correlations between benthic isoprene production and both temperature and light conditions, which explain a much larger proportion of isoprene variability than biomass. Additional biotic factors could explain part of the observed variation in estuarine emissions, including the role of microbial consumption [*Acuña Alvarez et al.*, 2009] and viruses [*Malin et al.*, 1998]. However, a more likely source of variation is the up- and down-regulation of isoprene synthesis in response to changes in environmental conditions, as further demonstrated by the results of our diel study, and supported by laboratory manipulations of microalgal growth conditions [*Shaw et al.*, 2010] and previous studies of coastal marine systems [*Broadgate et al.*, 2004].

[32] This study highlights the previously undervalued importance of coastal marine systems, particularly estuaries, to the isoprene budget; a factor which could have important implications for local weather patterns through the role of isoprene in atmospheric chemistry [*Pacifico et al.*, 2009]. The simple model employed here indicates an annual isoprene production rate of 681 μmol m^{-2} y^{-1} for intertidal sediment along the Colne. This falls above the range of 5 to 524 μmol m^{-2} y^{-1} for the open ocean based on chlorophyll modeling [*Palmer and Shaw*, 2005], and toward the upper end of the range of 52 to 3142 μmol m^{-2} y^{-1} based on atmospheric analysis [*Liakakou et al.*, 2007]. However, it remains more than two orders of magnitude below terrestrial fluxes [*Simeonidis et al.*, 1999]. Estuarine systems should form an important component of marine isoprene models, and further research on currently unstudied coastal systems should be pursued. Furthermore, if the ecophysiological benefits of isoprene synthesis shown in terrestrial plants [*Vickers et al.*, 2009] are also afforded to marine algae, the production rates observed in this study would play a significant role in the ability of algal assemblages to tolerate the high levels of environmental fluctuations present in temperate estuarine environments.

[33] In conclusion, the findings of this study suggest that intertidal benthic microphytobenthic communities are a main source of isoprene on soft shores of temperate estuaries. Variation in estuarine isoprene is partly driven by changes in algal biomass, but more importantly by the environmental factors temperature and light, suggesting that stress is an important driving factor in these environments. Tidal height was also shown to be a major driver of isoprene concentrations in the water column, although this appears to be a concentrating mechanism for isoprene entering the water column from the benthos. This fits with established terrestrial research highlighting the role isoprene plays in stress response [*Sharkey et al.*, 2008], and could prove important in shaping the composition of future estuarine communities in the face of increasing environmental perturbations [*Shaw et al.*, 2003].

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