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Primary production in nutrient-rich kettle holes and consequences for nutrient and carbon cycling

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Abstract

Kettle holes are often abundant within agriculturally used moraine landscapes. They are highly enriched with nutrients and considered hotspots of carbon turnover. However, data on their primary productivity remain rare. We analysed two kettle holes typical to Germany with common aquatic plant communities during one year. We hypothesised that gross primary production (GPP) rates would be high compared to other temperate freshwater ecosystems, leading to high sediment deposition. Summer GPP rates ($4.5\text{--}5.1\text{ g C m}^{-2}\text{ day}^{-1}$) were higher than those of most temperate freshwater systems, but GPP rates were reduced by 90% in winter. Macrophytes dominated GPP from May to September with emergent macrophytes accounting for half of the GPP. Periphyton contributed to most of the system GPP throughout the rest of the year. Sediment deposition rates were high and correlated with GPP in one kettle hole. In contrast, due to prolonged periods of anoxia, aerobic sediment mineralisation was low while sediment phosphorus release was significant. Our results suggest that kettle holes have a high potential for carbon burial, provided they do not fully dry up during warm years. Due to their unique features, they should not be automatically grouped with ponds and shallow lakes in global carbon budget estimates.

Introduction

Many studies have demonstrated that inland aquatic systems play an active role in global carbon (C) cycling (Cole et al., 2007; Battin et al., 2009; Raymond et al., 2011). The majority of these systems are shallow, lentic, small water bodies that can be defined by a surface area $<0.05 \text{ km}^2$ and a highly variable water depth, mostly resulting in a temporary water regime (Lorenz et al., 2016). Lentic small water bodies $<0.1 \text{ km}^2$ add up to a potential 20% of the global surface area of lakes due to their high abundance (Holgerson & Raymond, 2016). Staehr et al. (2011) concluded that an inverse relationship exists between metabolic rates (gross primary production (GPP) and respiration) and lake area as small water bodies receive larger quantities of allochthonous matter relative to lake volume and have higher probability of being heterotrophic than large ones (Sand-Jensen & Staehr, 2009). Organic C sequestration per unit area of sediment has been suggested to be at least an order of magnitude higher in small lakes than in larger lakes (Stallard, 1998; Dean & Gorham, 1998; Downing et al., 2008; Heathcote et al., 2016). In addition to lake area, C mineralisation (and consequently burial) in lake sediments is highly dependent upon oxygen (O_2) availability (Sobek et al., 2009). Isidorova et al. (2016) found that anaerobic conditions reduce C mineralisation by roughly 50% compared to aerobic respiration, often resulting in an enhanced C burial in lake sediments. Thus, primary production, through its contribution to C sequestration and O_2 availability in the water column, plays a crucial role in C burial in small, shallow aquatic systems and the overarching global C cycle.

Kettle holes (sometimes referred to as prairie potholes in North America) are a common small lentic small water body ($<1 \text{ ha}$) in northern Europe and North America. Most were formed following the last glaciation (about 12,000–10,000 years ago), when the delayed melting of ice blocks created depressions in the moraine landscape without outlets (Mitsch & Gosselink, 1993; Kalettka et al., 2001; Creed et al., 2013). Anthropogenic influences such as forest clearance and tillage also seem to have enhanced their development (Kalettka et al., 2001). In northeastern Germany, up to 300,000 kettle holes exist, comprising up to 5% of the arable land (Kalettka & Rudat, 2006). Most of these kettle holes are located within agricultural landscapes. Thus their nutrient concentrations strongly exceed those of shallow lakes of the region (Lischeid & Kalettka, 2012; Eigemann et al., 2016). This potentially promotes primary production (PP) and C turnover (Reverey et al., 2016). Recent case studies indicate that these kettle holes play a significant role in landscape greenhouse gas emissions (Premke et al., 2016). However, when calculating the C budgets of kettle holes, a detailed knowledge of ecosystem processes (PP, sedimentation and mineralisation) is needed. Measuring PP in kettle holes is problematic since the standard single-site diel O_2 technique (Staehr et al., 2010) provides unreliable estimates of whole-system GPP in small lakes (Brothers et al.,

2013a). A high degree of spatial heterogeneity in summertime diel O₂ curves (Van de Bogert et al., 2012) can occur in macrophyte-dominated water bodies where the benthic zone plays a larger role in whole-ecosystem GPP than phytoplankton (Brothers et al., 2013a, 2017). Furthermore, long-lasting O₂ depletion may occur in small aquatic systems (Baird et al., 1987; Prairie et al., 2002), rendering the diel O₂ curve technique impractical. Therefore, other approaches must be pursued to circumvent the problem.

In kettle holes with high nutrient concentrations, emergent, submerged and floating macrophytes are often abundant during the May to September growing season (Lischeid & Kalettka, 2012). In contrast to temperate eutrophic shallow lakes (that are often characterised by the occurrence of alternative stable states with either macrophyte or phytoplankton dominance e.g. Scheffer et al., 1993; Zimmer et al., 2016), phytoplankton rarely dominates in kettle holes during the macrophyte growing season (Lischeid & Kalettka, 2012). Kettle holes dominated by macrophytes and surrounded by reed stands are the most common kettle hole type among intensively used agricultural landscapes of northeastern Germany and are defined by Luthardt & Dreger (1996) as ‘fringe type’ kettle holes. Despite the abundance of fringe-type kettle holes within these landscapes, specific contributions of various primary producer groups (phytoplankton, periphyton, rooted and free-floating macrophytes) to C dynamics (including C sediment deposition and mineralisation) within these systems are poorly understood (Vis et al., 2007), even though these processes play a pivotal role in landscape C budgets.

In this study we applied a compartmental approach, calculating the contribution of phytoplankton, periphyton, floating, submerged and emergent macrophytes to determine whole-system GPP during one year in two typical temperate, nutrient-rich, fringe-type kettle holes in northeastern Germany. We hypothesised that summer time (macrophyte growing season) GPP in the kettle holes would be comparable to very productive, temperate eutrophic aquatic systems, due to the high abundance of macrophytes during this period. Outside the macrophyte growing season, we expected periphyton to contribute significantly to GPP due to the high colonisable surface area to volume ratio of these systems. In addition, we hypothesised that high GPP would result in high sediment deposition rates, but low sediment mineralisation rates due to significant periods of anoxia, common in such systems due to a high share of emergent macrophytes not releasing O₂ into the water.

Materials and methods

Study sites

Two kettle holes were selected from the Uckermark region in Brandenburg, northeastern Germany. A detailed description of the location and bathymetric maps are reported elsewhere (Nitzsche et al., 2016; Kleeberg et al., 2016). Kettle hole Kraatz (N 53°25'05'' E 13°39'48'') was surrounded by a few *Salix cinerea*, L. shrubs and populated by a mixture of submerged, emergent and floating macrophytes (Table 1). Kettle hole Rittgarten (N 53°23'22'' E 013°42'09''), situated 5 km southeast of Kraatz, was sheltered by a reed belt (*Phragmites australis* (Cav.) Trin. ex Steud.) and fully covered by non-rooted submerged (*Ceratophyllum submersum* L.) and floating (*Lemna minor* L., *Spirodela polyrhiza* (L.) Schleid) macrophytes during the summer months (Table 1). Both kettle holes belong to the most common vegetation type in German kettle holes (fringe type according to Luthardt & Dreger, 1996), which are commonly characterised by permanent or perennial flooding (Kalettka, 1996).

Table 1: General characteristics and water chemistry of sampled kettle holes from May 2013 to April 2014. Data represent annual means (from 12 monthly samples, except for dissolved manganese where n = 9).

Parameters	Kraatz	Rittgarten
Area (m ²)	1510	1453
Mean depth (m)	0.6 ± 0.3	1.22 ± 0.3
Water temperature (°C)	10.3 ± 6.1	9.1 ± 4.5
pH	7.1 ± 0.3	6.8 ± 0.3
Total nitrogen (TN, mg L ⁻¹)	0.9 ± 0.2	2.0 ± 0.6
Total phosphorus (TP, µg L ⁻¹)	116.3 ± 78.4	256.5 ± 221.6
Soluble reactive phosphorus (SRP, µg L ⁻¹)	27.9 ± 22.6	150.3 ± 172.7
Total dissolved nitrogen (TDN, mg L ⁻¹)	0.9 ± 0.2	1.8 ± 0.5
Dissolved silica (mg L ⁻¹)	0.4 ± 0.3	4.1 ± 1.0
Dissolved manganese (mg L ⁻¹)	0.2 ± 0.3	0.7 ± 0.6
Dissolved organic carbon (DOC, mg L ⁻¹)	10.1 ± 1.2	19.6 ± 2.2
Dissolved inorganic carbon (DIC, mg L ⁻¹)	23.5 ± 2.9	57.7 ± 6.7
Total organic carbon (TOC, mg L ⁻¹)	10.7 ± 1.6	20.5 ± 2.4
Total inorganic carbon (TIC, mg L ⁻¹)	25.1 ± 3.5	65.1 ± 10.3

Both kettle holes are surrounded by arable land and are heavily exposed to agricultural practices such as tillage and fertiliser addition, leading to high nutrient concentrations (Table 2). Both kettle holes are sheltered from strong winds (mean ± SE = 1.8 ± 0.01 m s⁻¹), with Kraatz located in a depression while Rittgarten surrounded by a dense reed belt. Neither of the kettle holes was observed receiving continuous surface runoff during the study period. Input of terrestrial particulate organic matter (POM) was limited to extreme winter weather events when there was no significant vegetation and

was observed to be higher in Kraatz than in Rittgarten due to sharper surrounding inclines (C. Hoffmann, pers. comm.), in addition to a potential POM input from the surrounding bushes.

Table 2: Sampled standing stock of biomass and coverage of emergent, submerged and floating plant species in in two kettle holes with different vegetation types in June 2013.

Kettle hole	Type of vegetation	Species	Sampled standing biomass (g dry weight m ⁻²)	Coverage (% kettle hole surface area)	Quadrat size used (m ²)
Kraatz	Emergent	<i>Carex acutiformis</i>	221.3 (± 54.7)	20	0.16
		<i>Sparganium erectum</i>	19.7 (± 2.0)	10	0.16
	Submerged	<i>Potamogeton natans</i>	24.5 (± 6.7)	20	0.36 m ³
		<i>Potamogeton acutifolius</i>	36.0 (± 5.3)	5	0.36 m ³
		<i>Polygonum amphibium</i>			
	Floating	<i>Lemna minor, Spirodela polyrhiza, Lemna trisulca</i>	4.6 (± 1.4)	1	0.09
			4.8 (± 0.5)	15	0.09
Rittgarten	Emergent	<i>Phragmites australis</i>	232.1 (± 2.2)	35	1
	Submerged	<i>Ceratophyllum submersum</i>	64.1 (± 6.1)	55	0.36 m ³
	Floating		45.0 (± 18.2)	70	0.09
		<i>Lemna minor, Spirodela polyrhiza</i>			

Measurements of physical parameters

Oxygen concentrations in the water column were measured every 30 min throughout the sampling period (May 2013 to April 2014) via a Yellow Springs Instruments monitoring probe (YSI; Xylem Inc., Yellow Springs, OH, USA) hanging initially at a depth of 1 m in the middle of the kettle hole and later raised to the middle of the water column when the water level dropped below 1 m. Due to a breakdown of the YSI at Kraatz, O₂ data were unavailable between August 29 and October 18. Five additional O₂ probes (MiniDOT loggers, PME, USA) were placed randomly in each kettle

hole to investigate spatial O₂ heterogeneity by recording O₂ concentrations and temperature at 30 min intervals from August 8th to October 17th, 2013.

Water level fluctuations were measured by water depth loggers (CS451 Pressure transducer, Campbell Scientific, USA) installed in the centre of the kettle holes. Water volume, area and mean water depth (Z mean) were calculated using water level fluctuations and tachymetric data collected in June 2013. In Rittgarten, global radiation (in W m⁻²) and wind speed (in m s⁻¹) were measured every 30 min at a weather station located directly by the kettle hole using a CMP3 pyranometer (Kipp and Zonen, Delft, The Netherlands) and a MeteoMS multisensor (ecoTech Bonn, Germany), respectively. Mean light attenuation (ϵ) was calculated by measuring light intensity captured by two Underwater Spherical Quantum Sensors (LI-193, LI-COR BioSciences, Lincoln, NE, USA) fixed vertically 0.5 m apart, measured from just below the water surface, then lowered gradually till the lower bulb hit the sediment. When the water levels dropped during summer, only 1–2 measurements were possible. Photosynthetically active radiation (PAR) at depth Z was calculated from global radiation (in W m⁻²) and light attenuation using the following formula:

$$I_z = I_0 \times e^{-\epsilon \times Z}, \quad [eq.1]$$

where I_z represents irradiance (in $\mu\text{mol m}^{-2}\text{s}^{-1}$) at depth Z and I_0 represents irradiance on the surface of the water.

Measurements of water chemistry parameters

Depth-integrated 2 L water samples were taken from the centre of the kettle holes every four weeks from May 2013 until April 2014, using a Limnos water sampler (LIMNOS, Turku, Finland). Water samples were filled in separate vials and transported in dark coolers to the laboratory, where a number of water chemistry parameters (listed in Table 2) were analysed following German standard procedures (DEV, 2009).

Phytoplankton gross primary production

Phytoplankton fluorescence and biomass (chl-*a*) were measured from monthly water samples. Fluorescence was measured on an aliquot of water using the Phyto-US measuring unit of a pulse amplitude modulated fluorometer (Phyto-PAM, Walz, Effeltrich, Germany) after a dark adaptation period of at least 15 min. Measurements were corrected by subtracting background fluorescence from kettle hole water filtered through 25 mm diameter Whatman Glass Fibre Filters (GF/F). Another aliquot of kettle hole water was filtered through 25 mm diameter Whatman GF/F filters to measure chl-*a* concentrations by High Performance Liquid Chromatography (HPLC, Waters, Milford, MA, USA) following the procedure described in Shatwell et al. (2012). Carbon and N

contents of phytoplankton were measured following filtration through pre-washed, pre-ashed MicroTech GravityFlo Filters (MGF) and analysed on a Vario EL Elemental Analyzer (Elementar Analysensysteme GmbH, Germany).

Phytoplankton GPP was estimated following Brothers et al. (2013a) using fluorescence-based rapid photosynthesis–light curves, phytoplankton chl-*a* concentrations, photosynthetically active radiation (PAR, calculated as 46% of global radiation) at water surface and light attenuation at every 10 cm depth, multiplied by the corresponding water volume at each depth. Daily rates were calculated by interpolating monthly chl-*a*, fluorescence and light attenuation values using linear relations between monthly samples.

Additionally, to test for potential GPP of phytoplankton in the absence of light restricting floating vegetation, we performed a manipulative experiment at Rittgarten in July 2014. Duckweed and hornwort cover was harvested using nets, thus facilitating the penetration of sunlight into the water column. Concentration of chl-*a* was measured in water samples taken before and two weeks after cover clearance. Simultaneous O₂ concentrations in the water column were monitored by the YSI probe.

Periphyton gross primary production

Periphyton was grown in situ on transparent polypropylene strips with textured surfaces (IBICO, GBC, Chicago, IL, USA) installed 10 cm below the water surface and subsequently every 50 cm till the sediment was reached. Four large (15 × 2 cm) and four small (4.5 × 1.3 cm) plastic strips from each depth were harvested every month and replaced by new ones. The large strips were transported to the laboratory in plastic cylinders deposited in dark and humid coolers, whereas the small ones were stored in 15 mL plastic tubes filled with filtered kettle hole water to avoid zooplankton grazing during transportation. Periphyton on the large strips was brushed off in the laboratory using a toothbrush and filtered kettle hole water. The suspension was then filtered onto GF/F and MGF filters to determine chl-*a* concentrations and C and N contents (as described above for phytoplankton). Periphyton on the smaller strips was dark adapted for at least 15 min prior to measuring rapid photosynthesis–light curves using a Phyto-PAM Emitter Detector Fiberoptics (EDF) unit. Kettle hole periphyton GPP was calculated following the equations described in Brothers et al. (2013a), using the calculated surface area available to epipelon (periphyton growing on sediment) and epiphyton (periphyton growing on submerged surfaces of macrophytes) in each system. Epipelon was assumed to grow on all water-covered surfaces within the kettle holes (determined by tachymetric techniques), whereas the surface area of macrophyte leaves (on which epiphyton could grow) was calculated following methods described in the subsequent section. As with

phytoplankton GPP calculations, daily biomass and light attenuation values were extrapolated using linear equations between monthly measurements.

Macrophyte gross primary production

We identified all macrophytes at the two sites to the species level and visually estimated the percent surface cover of each species to the nearest 5% during field surveys and via monthly aerial pictures. Macrophyte biomass in both kettle holes was sampled in the third week of June 2013, when standing stock is usually greatest based on previous observations and studies done on similar systems within the same region (e.g. Pätzig et al., 2012). We sampled each species at four random locations in each kettle hole that were fully covered with vegetation using quadrats of varying sizes (Table 1) depending on the growth form and species size. Submerged species were collected with a volumetric sampler ($V = 0.36 \text{ m}^3$) to allow for depth-integrated measurements. In the laboratory, we dried the biomass samples at 60°C for seven days to obtain dry weight (DW). Dried samples were ground and aliquots weighed into tin cups for C and N analysis (Vario EL Elemental Analyzer). Minimum standing stock was estimated to be negligible prior to May and after September (for submerged and floating macrophytes) or October (for emergent macrophytes), while maximum standing stock was achieved around the time of sampling in late June. Temporal fluctuations in standing stock and GPP (in $\text{g C m}^{-2}\text{day}^{-1}$) of each macrophyte species during their growth period (May to September/October) were calculated by fitting a polynomial curve that included the aforementioned minimum and maximum standing stock estimations and their C content on a DW basis. GPP was calculated by multiplying the maximum–minimum biomass by a gross production rate-to-harvest ratio of 1.5 for submerged and floating macrophytes (Best, 1982 and references within) and *P. australis* (Hocking, 1989), and was estimated for an active growing period of six months of the year (following observations).

We estimated the total leaf area (LA) of submerged surfaces on macrophytes (available for epiphyton colonisation) using the equation:

$$\text{LA} = \text{DW} \times A \quad [\text{eq.2}]$$

with DW as the dry weight in g and A as the area in $\text{cm}^2 \text{g}^{-1} \text{DW}$. Values of A are known to differ (by a range of 500–1500) among species (Filbin & Hough, 1983; DVWK, 1990; see Körner & Köhl, 1996). Submerged, highly branched species tend to have higher A values, while macrophytes with simple structures have low A values (Pettit et al., 2016). For this reason, grouping of morphologically similar plants has been shown to be a viable approach in the absence of measurements for particular species (Armstrong et al., 2003). Here, we used A values for *P. pectinatus* L. (Börner) ($A = 1068 \text{ cm}^2 \text{g}^{-1}$; Fischer &

Pusch, 2001), *P. richardsonii* (Benn) Rydb. ($A = 766 \text{ cm}^2 \text{ g}^{-1}$), *Ceratophyllum demersum* L. ($A = 427 \text{ cm}^2 \text{ g}^{-1}$; Armstrong et al., 2003) instead of *P. acutifolius* Link ex Roem. & Schult., *P. natans* L., and *C. submersum* L., respectively.

In Kraatz, emergent macrophytes such as the *Carex* spp. bushes and *Sparganium erectum* L. were not included in these calculations as the sharp water level decrease during the summer months led to these plants to be outside the submerged area, and their surface area therefore is unavailable for periphyton colonisation. In Rittgarten, a small portion (~20%) of *Phragmites australis* remained within the submerged area. To calculate the additional surface area provided by *P. australis* for periphyton colonisation, we measured density (within four random 1 m^2 quadrats) and average circumference of each stem. Colonisable reed surface area (CRSA) was then calculated as

$$\text{CRSA} = \text{reed density} \times \text{average circumference} \times \text{average water depth of submerged part} \quad [\text{eq. 3}]$$

Total areal GPP and aquatic (autochthonous) GPP calculations

In order to make broad comparisons with other studies, system GPP was calculated in two distinct manners: total GPP and aquatic GPP. Total GPP was estimated by summing the GPP of all primary producer groups, including the allochthonous production of emergent macrophytes (species uptaking atmospheric C), as well as the autochthonous production (assimilating aquatic C) of phytoplankton, periphyton, and submerged and floating macrophytes (the latter group is reported to utilise both sources of C; Filbin & Hough, 1985). For areal total GPP, we divided total GPP by the static kettle hole area (designated by the circumference at the top shore line), irrespective of water level fluctuations throughout the year. This step was necessary to ensure the inclusion of all emergent macrophytes that were likely connected to the water column via their roots, despite falling outside the water column boundaries aboveground when the water volume receded in the warm summer months.

Aquatic GPP (e.g. Hagerthey et al., 2010) was calculated by summing only autochthonous GPP values, thus excluding emergent macrophytes from these calculations. Alternatively, areal aquatic GPP was calculated by dividing the above value by the daily-varying kettle hole surface area, which was derived from daily measurements of water level fluctuations. Therefore, while total areal GPP gives a more accurate indication of overall C sequestration (both allochthonous and autochthonous) within the boundaries of the kettle hole, aquatic GPP calculations can be used to directly compare our calculations to gross aquatic production (GAP) rates in literature (Hagerthey et al., 2010), obtained using different methods.

Sediment deposition rates

Pairs of sediment traps, acrylic glass tubes 56 cm in height and 6 cm in diameter, were deployed in a north–south transect at three sites of each kettle hole. Each pair of traps was exposed on vertical tubing directly on the sediment surface and emptied biweekly between June to November 2013, and between April to June 2014. Given the brevity of the second sampling phase, we did not include these results in our statistical tests but still present them in our figures. The sedimentation rate was calculated as the mean for the three trap sites representing the mean pond-specific flux ($n = 6$). Since the downward flux of matter is closely coupled to the prevalent water level, the measured pond-specific rates were normalised to 1 m of water depth. A more detailed description of the method can be found elsewhere (Kleeberg et al., 2016a).

Sediment respiration

Aerobic sediment respiration (R) was determined based on O₂ depletion rates in the overlying water of sediment incubation cores. Four random sediment cores were taken each month using a sediment corer (inner diameter = 6 cm; Uwitec, Mondsee, Austria). The top 10 cm of the sediment (and the overlaying water) was then transferred at the field into transparent, acrylic incubation cores of 5.3 cm diameter and 30 cm length (total volume ~0.5 L). Incubation cores were closed with a rubber stopper, transported in a cooler to the laboratory, placed into a dark chamber and kept at in situ temperatures overnight. In order to avoid O₂ depletion, the cores were kept open overnight. The next morning, cores were closed with a gas tight stopper, equipped with a floating magnet and incubated for roughly 3–24 h, depending on the initial O₂ concentrations. The magnet was used to periodically mix the overlaying water column in order to avoid any stratification or the establishment of an O₂ gradient. Oxygen depletion (ΔO_2 in mg L⁻¹) in the overlaying water over time (Δt ; in hours) was measured every two or three hours using a needle-type O₂ microsensor (Optode, PreSens, Regensburg, Germany) inserted about 1 cm above the sediment through the septum. The O₂ sensor was connected to a micro-fibre optic O₂ m (Microx TX3, PreSens, Regensburg, Germany) to log O₂ concentrations.

Oxygen depletion rates were converted to C respiration rates using an empirical conversion factor of 0.85 (Graneli, 1979). Total C mineralisation rates were calculated as follows:

— — —

[eq. 4]

where V_w denotes the volume of the sediment overlaying water (in L), A_s is the sediment surface area (in m^{-2}) and MW represents the molecular mass of C and O_2 (in $g\ mol^{-1}$), respectively.

Statistical data treatment

Effects of water nutrient concentrations on aquatic GPP were tested by repeated measures ANOVA, after log transformation of the parameters that did not exhibit normality. Shapiro–Wilk and Bartlett tests were respectively used to confirm the normality and homogeneity of variances of the concerned parameters. Correlation between GPP and both sediment deposition rates and aerobic mineralisation rates were tested with Spearman's rank correlation coefficients. All statistical analyses and graphs were produced using R (R Core Team) version 3.2.2 and Origin Pro 8.5.

Results

Water level and chemistry

During the sample period, the water level in Kraatz dropped significantly from a mean depth of 1.2 to 0.4 m, and from 1.8 to 0.9 m in Rittgarten, decreasing the submerged area by 67 and 50%, respectively. Water chemistry parameters showed strong temporal variations in both kettle holes. Total nitrogen (TN) remained high (≥ 1.1 mg L⁻¹ in Kraatz and ≥ 2.3 mg L⁻¹ in Rittgarten) throughout the summer months but decreased slightly thereafter. Total P (TP) was highest in June in both kettle holes (Fig. 1) and notably a sharp increase in both TP and soluble reactive P (SRP) between May and August (especially in Rittgarten) coincided with the prevailing anoxic conditions in the water column (Fig. 1). Mean dissolved organic carbon was higher in Rittgarten than in Kraatz (Table 2) and was slightly higher within both kettle holes during the summer and autumn months before declining in winter. Both kettle holes froze for a period of about ten weeks between December 2013 and February 2014.

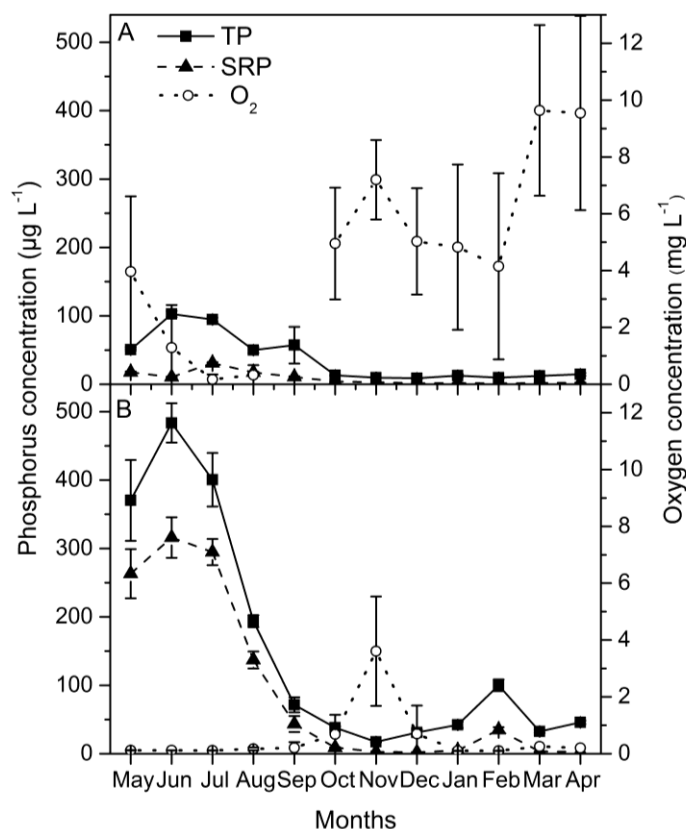


Fig. 1: Temporal fluctuations of total phosphorus (TP), soluble reactive phosphorus (SRP) concentrations (µg L⁻¹) and oxygen concentrations (mg L⁻¹) throughout the sampling period (May 2013 to April 2014) in two kettle holes **A** Kraatz and **B** Rittgarten.

Gross primary production

Annual total GPP was 956 and 914 kg C a⁻¹ in Kraatz and Rittgarten, respectively. Areal daily GPP rates averaged 1.77 ± 2.2 g C m⁻² day⁻¹ (mean \pm SD) in Kraatz and 1.83 ± 1.9 g C m⁻² day⁻¹ in Rittgarten. Macrophytes constituted a significant portion of the total production, accounting for 90 and 81% of the GPP in Kraatz and Rittgarten, respectively. Emergent macrophytes contributed nearly half of the total GPP in both kettle holes (Table 3). Periphyton comprised the majority of the remaining GPP, contributing 10% in Kraatz and 19% in Rittgarten (Table 3). Phytoplankton production was limited in both kettle holes (representing <1% of total annual GPP). During summer (peak macrophyte growing months; June–August) mean GPP rates were 5.1 ± 0.1 g C m⁻² day⁻¹ (mean \pm SD) and 4.5 ± 0.6 g C m⁻² day⁻¹ in Kraatz and Rittgarten, respectively (Fig. 2; Supplementary Table 1). System GPP rates dropped considerably throughout the remaining seasons (Fig. 2; Supplementary Table 1).

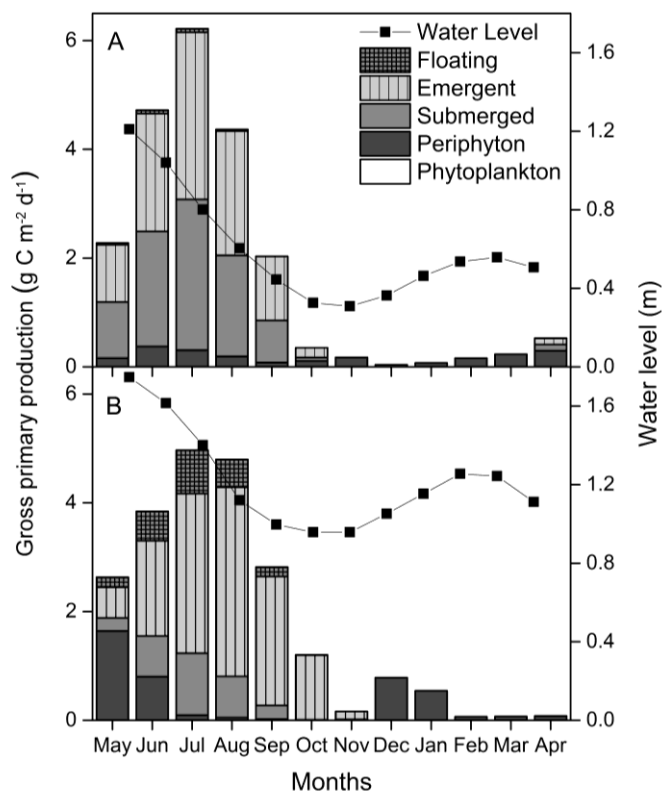


Fig. 2: Monthly gross primary production (GPP, g C m⁻² day⁻¹) including the contributions of different primary producer groups in two kettle holes: **A** Kraatz **B** Rittgarten.

A decline in water levels during the summer of 2013 reduced the surface area available to aquatic GPP calculations (Fig. 2). Annual aquatic GPP averaged 1.2 ± 1.3 g C m⁻² day⁻¹ in Kraatz and 1.2 ± 1.4 g C m⁻² day⁻¹ in Rittgarten (Table 3). Aquatic GPP rates were highest during summer months and averaged 3.2 ± 0.7 and 2.8 ± 0.5 g C m⁻² day⁻¹ in the two kettle holes, respectively (Supplementary Table 1). Despite the differences in

water nutrient concentrations between the kettle holes (Table 2), only SRP was shown to effect system GPP in both kettle holes (repeated measures ANOVA, $P < 0.05$, Supplementary Table 2).

Table 3: Mean total (allochthonous + autochthonous) and aquatic (only autochthonous) areal gross primary production ($GPP \pm SE$ in $mg\ C\ m^{-2}\ d^{-1}$) rates of the different primary producer groups and their contribution to overall GPP inside parentheses (in percent) in the two studied kettle holes from May 2013 to April 2014.

	Kraatz		Rittgarten	
	Total	Aquatic	Total	Aquatic
Phytoplankton	0.5 ± 0.2 (0.04 %)	0.5 ± 0.2 (0.01 %)	0.7 ± 0.02 (0.05 %)	0.7 ± 0.2 (0.1 %)
Periphyton	184 ± 30 (11 %)	284 ± 39 (23 %)	348 ± 146 (19 %)	533 ± 200 (43 %)
Submerged macrophytes	727 ± 288 (41 %)	910 ± 354 (75 %)	261 ± 115 (14 %)	410 ± 179 (33%)
Floating macrophytes	17.3 ± 7.6 (1 %)	19.9 ± 8.5 (2 %)	185 ± 80 (10 %)	289 ± 124 (24 %)
Emergent macrophytes	837 ± 319 (47 %)	---	1036 ± 372 (57%)	---

Temporal dynamics of different primary producer groups

Phytoplankton GPP rates were highest in May and June 2013 in both kettle holes, after which they gradually decreased. Periphyton GPP was relatively uniform in Kraatz. In contrast, GPP in Rittgarten was highest in May before declining sharply during summer, likely in response to shading by duckweed (Fig. 2). Periphyton areal GPP increased during winter, but a declining water level led to lower colonisation area (Fig. 2). Periphyton GPP contributed 43% to the annual aquatic GPP in Rittgarten and contributed to the majority of the kettle hole's total GPP outside of the macrophyte growing season.

Daily macrophyte (floating, submerged and emergent) GPP between May and October (macrophyte growth season) was $3.1 \pm 2.1\ g\ C\ m^{-2}\ day^{-1}$ (mean \pm SD) in Kraatz and $2.9 \pm 1.7\ g\ C\ m^{-2}\ day^{-1}$ in Rittgarten. Among the submerged macrophytes in Kraatz, *Potamogeton natans* and *P. acutifolius* contributed most to system GPP (Table 1). *Carex acutiformis* Ehrh., *Sparganium erectum* represented the greatest share of emergent macrophytes (Table 1), but following the initial decline in water levels in early July, they occupied an area beyond the aquatic zone. Floating plants (Table 1) altogether covered 16% of the surface area of Kraatz. In contrast, duckweed (a mixture of *Lemna minor* L., *Spirodela polyrrhiza* L.) covered 100% of the water surface of the other kettle hole. *Ceratophyllum submersum* L. formed a 10 cm dense mat beneath the duckweed, covering roughly 55% of the kettle hole area. At their peak, the submerged parts of the

macrophytes created additional surface area for periphyton colonisation, amounting to 7710 m² in Kraatz and 6880 m² in Rittgarten.

Manipulative experiment of duckweed harvesting

The manipulative experiment in Rittgarten resulted in an increase in phytoplankton chl-*a* concentration from 3.2 (SD: ± 0.1) $\mu\text{g L}^{-1}$ to 45 (± 0.9) $\mu\text{g L}^{-1}$ two weeks after the harvest of the duckweed and *Ceratophyllum* cover. Simultaneously, the water column transitioned from anoxia to >30% O₂ saturation. During that period, phytoplankton GPP increased 92% (up to 0.02 mg C m⁻² day⁻¹). Duckweed returned to cover 100% of the water surface area three weeks after the manipulative experiment and subsequently phytoplankton chl-*a* concentration reverted to 5 $\mu\text{g L}^{-1}$.

Sediment deposition

Sediment deposition rates from June to November 2013 amounted to 0.84 g C m⁻² day⁻¹ in Kraatz (range: 0.24–3.09 g C m⁻² day⁻¹) and 1.88 g C m⁻² day⁻¹ in Rittgarten (range: 0.5–3.77 g C m⁻² day⁻¹) (Fig. 3). The highest sediment deposition rates were recorded in June in Kraatz and in August in Rittgarten. Sediment deposition rates showed a strong correlation to GPP in Rittgarten (Spearman $\rho = 0.89$, $P = 0.034$), but not in Kraatz (Spearman $\rho = 0.49$, $P = 0.36$). From June until the end of November 2013, the cumulative mass of C settled represented 63% of the organic C produced by GPP in Rittgarten and 29% in Kraatz (Fig. 4).

Aerobic sediment mineralization

Aerobic sediment mineralisation rates ranged between 0.1 to 0.15 g C m⁻² day⁻¹ in Kraatz and 0.05 to 0.09 g C m⁻² day⁻¹ in Rittgarten (Fig. 3). The highest rates were recorded in December in Kraatz and in June in Rittgarten. During several summer months sediment respiration (SR) measurements using dissolved O₂ were not possible due to the prevailing anoxia above the sediments during these months. Aerobic mineralisation rates were not correlated to GPP ($P = 0.25$) or sedimentation rates ($P = 0.1$) (Fig. 5).

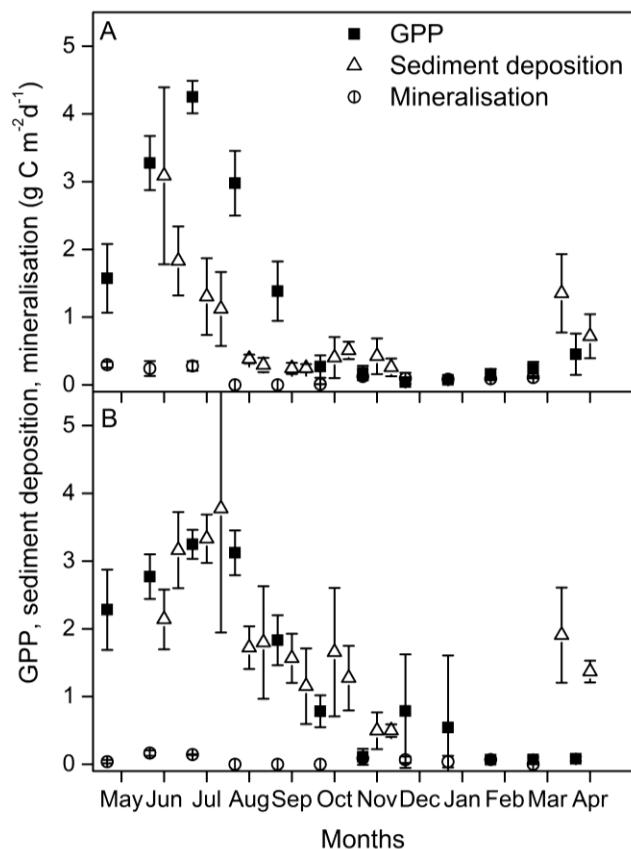


Fig. 3: Temporal variations of total gross primary production (GPP), sediment deposition rates and sediment aerobic mineralisation rates in two kettle holes: **A** Kraatz and **B** Rittgarten from May 2013 to April 2014.

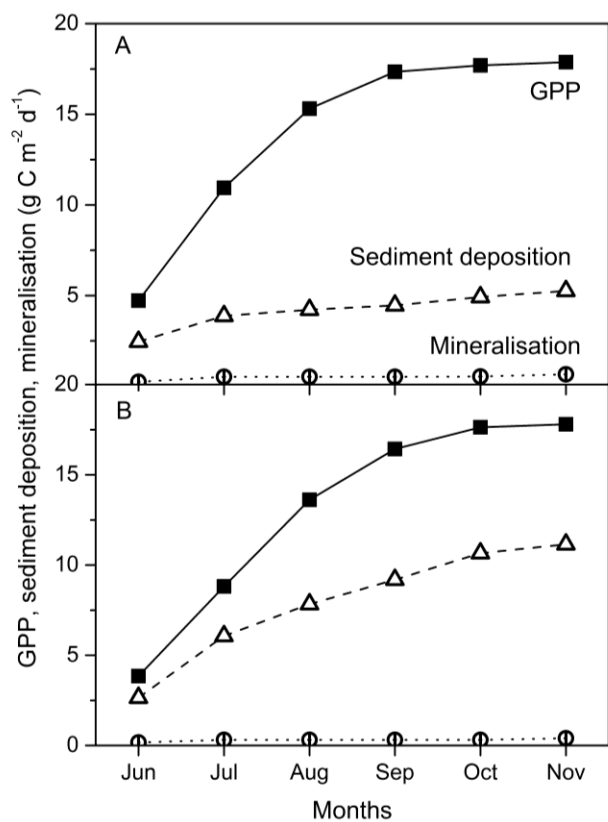


Fig. 4: Cumulative gross primary production (GPP) vs. cumulative sedimented material in **A** Kraatz and **B** Rittgarten from June to November 2013.

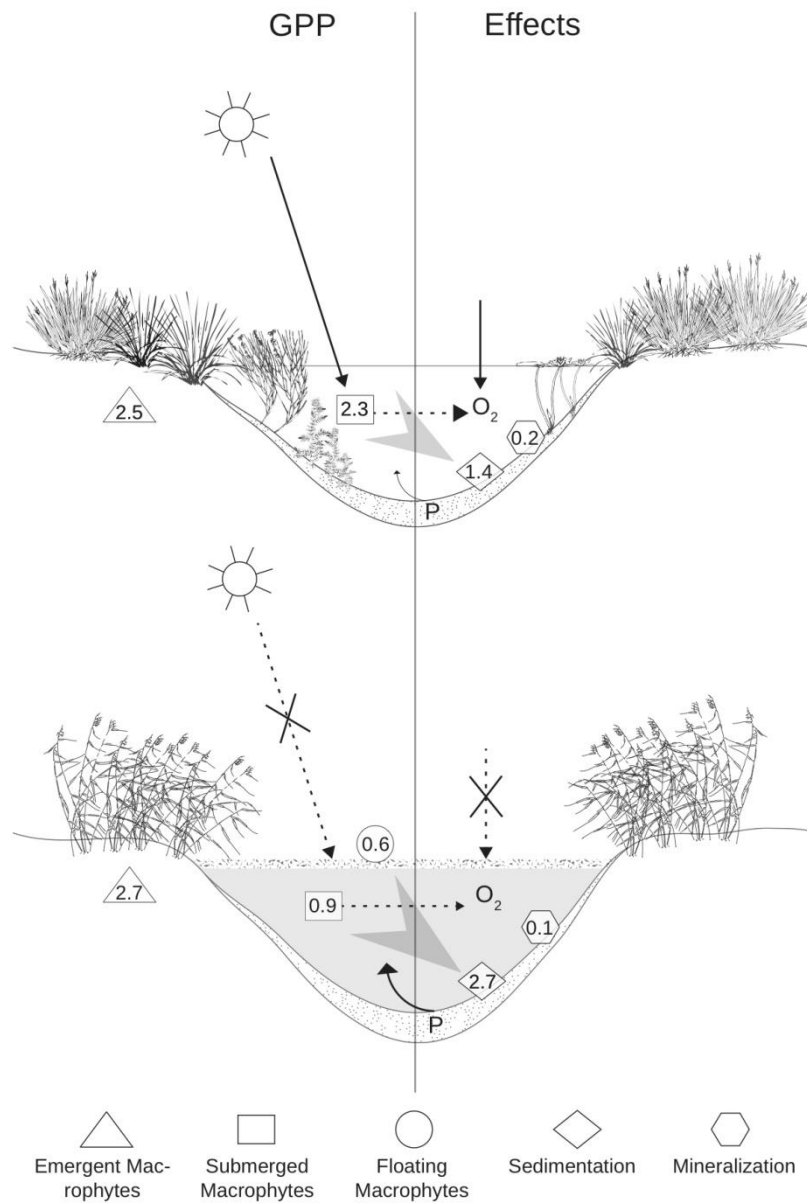


Fig. 5: Gross primary production (GPP) of the different primary producer groups during peak summer months (June to August) and their cascading effects on phosphorus (P) release from sediments and carbon sediment deposition and mineralisation (all units in $\text{g C m}^{-2} \text{ day}^{-1}$) in **A** Kraatz and **B** Rittgarten. Phytoplankton and periphyton constituted $<10\%$ of community GPP in the summer and hence were not shown here. Triangles represent emergent macrophyte GPP, rectangles represent submerged macrophyte GPP and circles depict floating macrophyte GPP.

Discussion

Summer daily GPP rates of our nutrient-rich, temperate kettle holes were high (Fig. 5) and comparable to the most productive natural eutrophic temperate freshwater ecosystems (Fig. 6). Emergent macrophytes dominated in the summer and accounted for about half of the annual GPP in both systems (47% in mixed vegetation and 57% in full duckweed cover). The duckweed cover and related anoxia in Rittgarten led to a strong redox-controlled P release from the sediments. Furthermore, summer sediment deposition rates were high and were strongly correlated to GPP in Rittgarten. Despite the availability of organic material, aerobic sediment mineralisation was low in both kettle holes, but specifically in Rittgarten due to prolonged periods of anoxia. Thus, the type of primary producers notably affected nutrient cycling and organic C processing, highlighting the structuring role of the plant communities on biogeochemical processes in the studied kettle holes (Fig. 5). The relatively high temporal resolution of this investigation, a rarity when looking at available literature, make it a valuable contribution to understanding how primary producers affect C dynamics and the uniqueness kettle holes pose compared to other aquatic ecosystems.

Comparison of kettle hole GPP to other systems

Summer total and aquatic GPP rates of the kettle holes were higher than those reported from other natural temperate lakes and ponds (Hanson et al., 2003; Hoellein et al., 2013; Supplementary Table 3). Due to lower GPP rates recorded throughout the winter months, annual GPP rates were equal or slightly higher than rates reported from similar pothole systems (Badiou et al., 2011; Euliss et al., 2006). Wetland GPP rates have previously been reported to be within the same range as those in our kettle holes (Buffam et al., 2011) or slightly higher (Reeder, 2011; Wagle et al., 2014). In contrast, several studies measured lower GPP values in larger lakes (see Supplementary Table 3: Carpenter et al., 2005; Coloso et al., 2008; Sand-Jensen & Staehr, 2009; Van de Bogert et al., 2012). However, the different methods used for measuring primary production (including C, DIC consumption, O₂ and fluorescence) and the different units used to report them (i.e. as O₂ production or C fixation) render comparisons between studies difficult. Additionally, depending on the method used, reported values either pertain to GPP, net ecosystem production (NEP), or even, in case of ¹⁴C measurements, to a value that lies in between GPP and NEP. Most studies on aquatic GPP have relied on O₂ probes or bottle incubations, making them phytoplankton-centric and excluding the contribution of emergent and floating macrophytes, while studies that adopt a compartmental approach (e.g. Wetzel, 1964; Liboriussen & Jeppesen, 2003; Blindow et al., 2006; Vis et al., 2007; Domine, 2011; Brothers et al., 2013a) are few.

In our study, the use of multiple O₂ probes revealed a high degree of spatial heterogeneity in O₂ saturation levels (data not shown). However, we were unable to effectively estimate ecosystem metabolism due to extended periods of anoxia or hypoxia throughout the water column. In order to account for high spatial heterogeneity of primary production within these systems—likely a product of nutrient-rich, shallow aquatic ecosystems featuring emergent, submerged and floating vegetation (Hanson et al., 2008; Van de Bogert et al., 2012) and extended periods of anoxia (Baird et al., 1987)—we recommend a compartmental approach when determining metabolic rates. A compartmental approach both estimates the contribution of each primary producer group to overall system GPP and avoids limitations pertaining to O₂ unavailability.

Nonetheless, the method is not without limitations. It is laborious, requires frequent sampling efforts and thus does not allow for a high temporal resolution in contrast to in situ continuous measurements. Estimating total system GPP relies on the summation of singular measurements of all PP groups, further increasing uncertainty by incorporating more random and variable measurement error. Standard deviation between sampled replicates of phytoplankton, periphyton and even macrophytes (Table 3) were found to be reasonably low. Instead, the greatest uncertainty within our GPP calculations lies within the biomass to GPP conversion factor chosen while calculating macrophyte GPP. This factor varies greatly (1.2–2.6) within reported literature (Westlake, 1982 and references within) depending on macrophyte species, season, water and air temperatures, light availability, and state of decay of the plants. In our GPP calculations, we used a conservative biomass to GPP conversion factor of 1.5 and thus might have represented the lower range of GPP estimates from these kettle holes. Another factor of uncertainty lies in macrophyte biomass to leaf area conversion rates (needed to estimate periphyton colonisable area), which can differ in a threefold range among species (Filbin & Hough, 1983) but also depending on environmental conditions (Spence & Chrystal, 1970). Nonetheless, in our particular study, given the relatively low periphyton biomass during the macrophyte growing season in both kettle holes (and thus its very minor contribution to system summer GPP) this particular uncertainty is not significantly pronounced with regards to the overall GPP calculations.

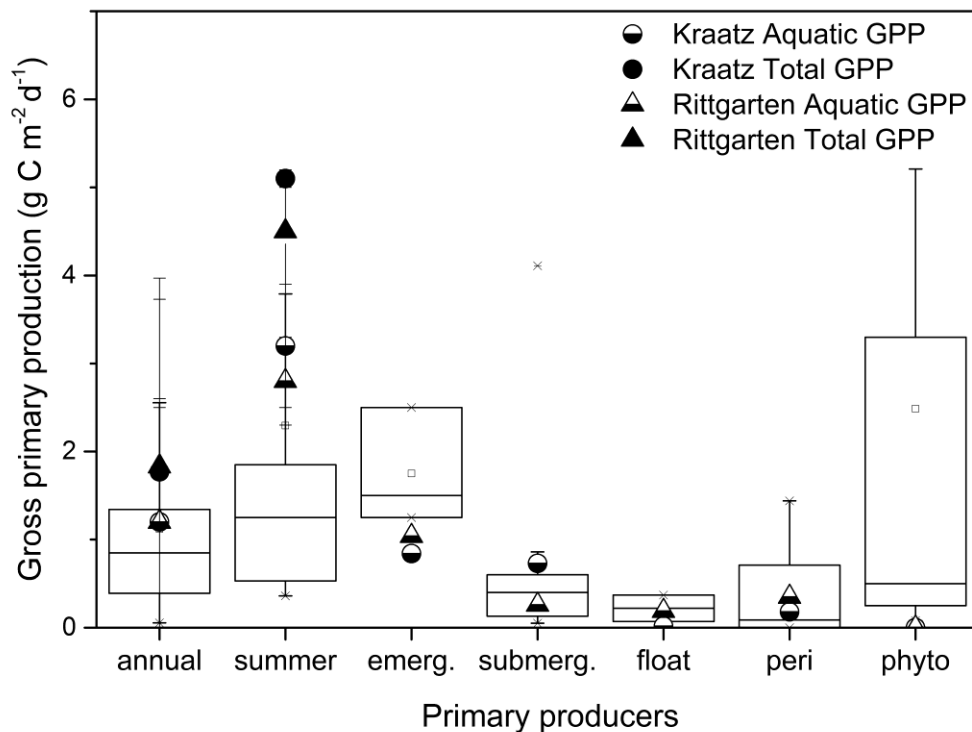


Fig. 6: Annual and summer total (allochthonous + autochthonous) and aquatic (autochthonous) gross primary production (GPP) rates, in addition to annual GPP rates from distinct primary producer groups (emergent, submerged and floating macrophytes, periphyton and phytoplankton) measured in two eutrophic kettle holes (Kraatz and Rittgarten) compared to equivalent GPP rates from other freshwater, temperate shallow systems previously reported in literature (boxplots). Data used from literature are detailed in supplementary Table 3.

Contribution of different primary producers to total GPP

Among different autotroph groups, emergent macrophytes contributed most to GPP in our kettle holes, a pattern that has also been observed within small temperate aquatic systems and wetlands (Fig. 6; Supplementary Table 3). Despite minimal direct gas exchange with the water column, emergent macrophytes are an important metabolic component of aquatic ecosystems, influencing the availability of organic C and nutrients to other aquatic primary and secondary producers (Wetzel, 2001). GPP of emergent macrophytes is rarely incorporated into terrestrial C balances, and excluding their contribution from aquatic C balances can lead to significant underestimation of C sequestration at the landscape scale (Abril et al., 2014).

Submerged macrophytes are usually less productive than emergent plants, mostly due to effects of self-shading, as well as shading by periphyton, phytoplankton

and other macrophytes (Wetzel, 2001). In our study, submerged macrophytes still represented a substantial fraction of the total GPP in Kraatz (Table 3). As with emergent macrophytes, herbivory on submerged macrophytes often leads to underestimated productivity rates. Wetzel (2001) reports that for *C. submersum*, true production rates can be three times greater than those estimated from maximum biomass values. We applied a conservative factor of 1.5 to calculate GPP from maximum standing crop and thus may have underestimated the production of some submerged macrophytes. However, our GPP values are still comparable to those in the literature (Fig. 6; Supplementary Table 3). In contrast, floating macrophytes constituted a rather small share of the total GPP in both kettle holes (Table 3). Despite this, they had a very significant impact on the nutrient and C dynamics, as described below.

Periphyton was the major contributor to system GPP during periods when the influence of macrophyte shading was negligible. Periphyton biomass in Rittgarten was more than double that of Kraatz, except during periods of complete cover by duckweed. Although potential periphyton colonisation area increased due to the spread of the submerged macrophytes, shading (and probably grazing) limited its production in summer. Annual periphyton GPP rates were within the same range or higher than those reported for other temperate water bodies (Fig. 6; Supplementary Table 3). In contrast, phytoplankton GPP rates in our kettle holes were lower than rates reported from temperate eutrophic shallow lakes (Fig. 6; Supplementary Table 3), indicating the minor role that they play in kettle holes (Table 3). Despite the high availability of nutrients, this was expected due to the low water depth of the kettle holes, shading and potentially allelopathy by macrophytes. Although not measured, the grazing potential on phytoplankton and periphyton by zooplankton and invertebrates was likely high due to the absence of fish (Jones & Sayer, 2003). The higher areal phytoplankton GPP and biomass in Rittgarten can be partially attributed to the initial 48% greater mean depth of that kettle hole compared to Kraatz. The low contribution of phytoplankton to aquatic GPP explains the lack of correlation between phytoplankton chl-*a* concentrations and aquatic GPP in the kettle hole with mixed vegetation. Shading effects of floating vegetation on phytoplankton were verified with the manipulative experiment in Rittgarten wherein the removal of duckweed and *Ceratophyllum* led to a brief phytoplankton bloom and the oxygenation of the water column. On the longer term, however, grazing by zooplankton might have also played a significant role in limiting phytoplankton production and biomass.

Primary production drives nutrient cycling, sediment deposition and benthic mineralisation rates

The effects of a given primary producer group on a system's nutrient and C dynamics were not proportional to the group's contribution to total GPP. Free-floating

macrophytes (duckweed) in Rittgarten constituted only 9% of the system's total GPP but triggered substantial cascade effects on several ecosystem processes. A full surface cover of floating plants impeded light penetration into the water column, limiting the growth of planktonic, epiphytic and epipelagic algae, and resulting in extended periods of anoxia. In addition, we calculated that a potential O_2 surface influx of $33.5 \text{ g } O_2 \text{ m}^{-2}$ was blocked by the full duckweed cover for roughly 90 days between June to September (Fig. 4). A strong increase in SRP concentrations in Rittgarten (Fig. 4; Kleeberg et al., 2016b) was likely due to the release of iron-bound P from settled matter and surface sediments, which can result from hypoxic or anoxic conditions (Gächter & Müller, 2003; Kleeberg et al., 2013). Given the steady-state conditions and assuming that there was no high external P input into the system during the same period, the increase in SRP concentrations is attributed to the release of P from settled matter and the surface sediments. After the disappearance of the floating vegetation during autumn and the subsequent re-oxidation of the water column (Fig. 4), TP and SRP concentrations decreased. Kleeberg et al. (2016b) reported that the sedimentary molar Fe:P ratio, an indicator of P mobility, was similar in both kettle holes. Nevertheless, as indicated by the molar S:Fe ratio in surface sediments (0–2 m) there was a much lower Fe availability for P binding in Rittgarten (S:Fe = 2.33) than in Kraatz (S:Fe = 0.06). At a S:Fe ratio <1.5, vivianite, an iron phosphate mineral ($Fe_3(PO_4)_2 \cdot 8H_2O$), can be formed (Rothe et al., 2015). In Rittgarten, O_2 depletion, exacerbated by shading, led to exceedance of this threshold and promoted effective sulphate reduction (Kleeberg et al., 2016a), consequently leading to the formation of insoluble iron sulphides (FeS_x). Thus, the duckweed dominance represents a self-stabilising mechanism, achieved through increasing P availability via a dense surface covering that directly lowers the O_2 flux from the atmosphere as well as indirectly by limiting O_2 production by submerged primary producers through shading. The establishment of a P-rich water column favours the annual re-occurrence of duckweed, resulting in a positive feedback between P availability and duckweed cover (Scheffer et al., 2003). Duckweed dominance in Rittgarten thus potentially represents a stable state (Scheffer et al. 2003) that is in contrast to patterns exhibited in Kraatz, where short periods of anoxia and low TP concentrations were prevalent.

Cumulative deposited material remained lower than cumulative GPP from June to November (Fig. 4) pointing to a low input of particulate organic matter by erosion or aeolian transport from the terrestrial catchment during this period. A discrepancy between cumulative GPP and sediment deposition rates in Kraatz might be explained by a lower probability of the prevailing species being caught in our traps and the later senescence of emergent macrophytes (6–12 months), while duckweed and *Ceratophyllum* mats were mainly senescing in the measuring period (within 1–3 months) (Twilley et al., 1985). This might also explain the differences in the peaks of aerobic mineralisation

occurring in June and December in Rittgarten and Kraatz, respectively. Regardless, sediment deposition rates in both systems are high compared to other aquatic systems (Ferland et al., 2014). The effects of primary producers on O₂ availability may also influence C burial rates, which have been shown to be high under anoxic conditions (Bastviken et al., 2004; Brothers et al., 2013b; Isidorova et al., 20156). However, in our study, the low C degradation by aerobic mineralisation in Rittgarten was likely compensated by higher methane (CH₄) emissions due to anaerobic C degradation, especially within the reed belt (C. Lisboa, pers. comm.). During the mostly anoxic months of full duckweed cover (May–September), CH₄ emission, measured on a monthly basis by a greenhouse gas analyser (ABB—Los Gatos Research, San Jose, CA, USA), averaged $0.21 \pm 0.1 \text{ g C m}^{-2} \text{ day}^{-1}$ (mean \pm SD) above the water column and $0.52 \pm 0.3 \text{ g C m}^{-2} \text{ day}^{-1}$ above the reed belt. During the same period, CH₄ emissions were lower in Kraatz, amounting to $0.14 \pm 0.1 \text{ g C m}^{-2} \text{ day}^{-1}$ (mean \pm SD) and $0.25 \pm 0.2 \text{ g C m}^{-2} \text{ day}^{-1}$ above the water column and surrounding emergent macrophytes, respectively. Anaerobic mineralisation is reported to contribute significantly to C loss from small aquatic systems (Holgerson, 2015). Regardless, aerobic and anaerobic mineralisation combined still do not add up to GPP or sedimentation rates within these kettle holes, indicating a high potential for C burial, if current conditions prevail.

Kleeberg et al. (2016b) showed lower burial rates in Rittgarten within the past century compared to Kraatz. We assume that this result mainly stems from a higher frequency of drying up of Kraatz during the last century. The assumed lower sediment deposition and higher mineralisation rates of buried material during the drying–rewetting period were supported by the molar ratio of the redox-sensitive elements Fe and Mn over the last 100 years from dated sediment cores (Kleeberg et al., 2016b). The data show a stronger variability in Rittgarten (Fe:Mn 86.1 ± 15 , $n = 622$) while being more constant in Kraatz (Fe:Mn 72.9 ± 4.9 , $n = 1022$), which did not dry up due to its location in a deeper depression. Hydroperiod (Rittgarten dries more frequently during summer than Kraatz) may also contribute to lower species richness in Rittgarten, since differences in nutrient profiles alone are unlikely to explain dissimilar plant community structure. Given the laborious methodology involved, our study focused only on two kettle holes. There is a need for further studies of kettle holes with similar characteristics (water levels and fluctuations) in order to more reliably isolate the effects of differences in plant community type. Regardless, this study provides valuable insight into C dynamics within these understudied systems and, in agreement with Carpenter (1989), we believe obtaining ecological knowledge about whole ecosystems is a valid approach even in the absence of sufficient replication.

In conclusion, our investigation revealed that nutrient-rich kettle holes in temperate moraine landscapes have relatively high GPP rates during the summer

months when they are dominated by emergent plants combined with either mixed or floating vegetation. Primary production produced strong cascading effects on temporal nutrient and C dynamics. It directly affected sediment C deposition, governed the availability of O₂ in the water column (through direct release from primary producers or limiting flux to and from the atmosphere by floating macrophytes) and thus indirectly impacted the aerobic mineralisation rates and phosphorus concentrations in the water column. All these processes combined also govern C burial in and greenhouse gas emissions from these kettle holes. Due to our limited sample size, more studies are needed to corroborate these results. Further studies involving kettle holes of different classifications and plant community types are also needed to determine the effects of these different groups on system GPP and C dynamics. Long-term data incorporating the frequency of dry periods and C loss by mechanisms such as CH₄ evasion would be helpful to determine landscape-scale C budgets in areas with abundant kettle holes. With projected lower future precipitation for the region (Germer et al., 2011) and consequently higher frequency of drying up, C burial potential of the kettle holes may sharply decrease (Reverey et al., 2016), leading to higher greenhouse gas emissions.

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