Extremophiles (2011) 15:597–609
DOI 10.1007/s00792-011-0390-3

ORIGINAL PAPER

Interactions between P-limitation and different C conditions on the fatty acid composition of an extremophile microalga

Elly Spijkerman · Alexander Wacker

Received: 25 May 2011 / Accepted: 4 July 2011 / Published online: 6 August 2011

Abstract The extremophilic microalga *Chlamydomonas acidophila* inhabits very acidic waters (pH 2–3.5), where its growth is often limited by phosphorus (P) or colimited by P and inorganic carbon (CO_2). Because this alga is a major food source for predators in acidic habitats, we studied its fatty acid content, which reflects their quality as food, grown under a combination of P-limited and different carbon conditions (either mixotrophically with light + glucose or at high or low CO_2, both without glucose). The fatty acid composition largely depended on the cellular P content: stringent P-limited cells had a higher total fatty acid concentration and had a lower percentage of polyunsaturated fatty acids. An additional limitation for CO_2 inhibited this decrease, especially reflected in enhanced concentrations of 18:3(9,12,15) and 16:4(3,7,10,13), resulting in cells relatively rich in polyunsaturated fatty acids under colimiting growth conditions. The percentage of polyunsaturated to total fatty acid content was positively related with maximum photosynthesis under all conditions applied. The two factors, P and CO_2, thus interact in their effect on the fatty acid composition in *C. acidophila*, and colimited cells P-limited algae can be considered a superior food source for herbivores because of the high total fatty acid content and relative richness in polyunsaturated fatty acids.

Keywords Acidophilic algae · Cellular P quota · *Chlamydomonas acidophila* · Chlorophyceae · Colimitation · CO_2 · Fatty acid composition · Food quality · Glucose · Mixotrophy · Photosynthesis · Phytoplankton · Phosphorus limitation

Abbreviations

Alpha (α)	Initial slope of the photosynthesis–irradiance curve
ANCOVA	Analysis of covariance
Beta (β)	Slope at high irradiances that describes photo-inhibition
DGDG	Digalactosyldiacylglycerol
FA	Fatty acid
I	Actinic light intensity
$K_{m(CO2)}$	Half saturation constant for CO_2 uptake
μ	Growth rate
MUFA	Monounsaturated fatty acid
Mixotrophy	Growth on glucose and light
P	Phosphorus
Pr	Photosynthetic rate
P_{max}	Maximum photosynthetic rate
PUFA	Polyunsaturated fatty acid
Q_p	Cellular P quota
R_d	Dark respiration rate
SFA	Saturated fatty acid

Communicated by A. Oren.

Electronic supplementary material The online version of this article (doi:10.1007/s00792-011-0390-3) contains supplementary material, which is available to authorized users.

E. Spijkerman (✉)
Department of Ecology and Ecosystem Modelling,
University of Potsdam, Am Neuen Palais 10,
14469 Potsdam, Germany
e-mail: spijker@uni-potsdam.de

A. Wacker
Department of Theoretical Aquatic Ecology,
University of Potsdam, Am Neuen Palais 10,
14469 Potsdam, Germany
e-mail: wackera@uni-potsdam.de

Introduction

Phosphorus (P) is an essential nutrient for algal growth and it is often the growth-limiting nutrient for the phytoplankton in extremely acidic lakes (pH 2.3–3.4; Spijkerman 2008a). One of the responses to a P-limitation in plants and algae is the membrane lipid remodelling: upon P-deficiency, a significant portion of membrane phospholipids is replaced by non-P galactolipids and sulfolipids (Andersson et al. 2003; Van Mooy et al. 2009). For example, in oat 70% of the plasma membrane phosphoglycerolipids were replaced by the galactolipid digalactosyldiacylglycerol (DGDG) when cultivated under severe phosphate limitation (Andersson et al. 2003). Although the fatty acid (FA) composition of lipids differs between different algal species (Dijkman and Kromkamp 2006), membrane lipid classes in the green alga *Chlamydomonas reinhardtii* typically are characterized by unsaturated FAs such as 16:3, 16:4, 18:1, 18:2, 18:3 and 18:4 (Giroud et al. 1988). More specifically, DGDG in *C. reinhardtii* consisted for 96% of C18-FAs (predominantly 18:1(9), 18:2(9,12) and 18:3(9,12,15)) on the C-1 position of the glycerol moiety and 97% of C16-FAs (mainly 16:0) on the C-2 position (Giroud et al. 1988). In one study on the lipid and FA composition of a *Chlamydomonas acidophila* isolate from an acidic volcanic lake of Japan, the content of DGDG was too low to be analysed among the major lipids (8 mol%; Yamamoto et al. 1998). These authors analysed monogalactosyl diacylglycerol in the species grown at pH 3 (14 mol%), which consisted of 16:0 for 41%, 18:1(9) for 23% and 18:3(9,12,15) for 24% FAs and stated that these three FAs were also the major components of the lipid DGDG. As a result of the changes in lipid class composition, P-limited phytoplankton will have a different FA composition than the P-saturated (Ahlgren et al. 1997). For example, the green alga *Scenedesmus quadricauda* had a a lower percentage of polyunsaturated fatty acids (PUFAs) to total FAs at P-limited than at P-replete conditions, mainly as a result of the lower content of 18:3(9,12,15) (Ahlgren et al. 1998). Additionally, under P-limitation cell division rates decrease, although photosynthetic rates are much less reduced. This leads to an accumulation of carbon, which might be stored in the form of triacylglycerols that are rich in saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA; Guschina and Harwood 2009). Changes in the cellular P quota and FA composition in the phytoplankton modified the food quality for herbivores (Müller-Navarra 1995; Villar-Argaiz et al. 2009) and a decreased PUFA content in algae will directly or indirectly hamper growth and health of fish (Tocher 2003; Arts and Kohler 2009).

In many lakes, growth of the phytoplankton is not only P-limited, but possibly colimited by several nutrients (Elser et al. 2007; Sterner 2008) and for herbivores a colimitation by P and/or FAs and sterols seems plausible (Martin-Creuzburg et al. 2009; Lukas et al. 2011). Therefore, studies on the changes of FA composition in algae cultured under colimiting conditions are ecologically relevant. A study on extremely acidic lakes (pH 2.3–3.4) revealed that growth of the phytoplankton, and specifically *C. acidophila*, was P-limited (Spijkerman 2008a). Surface strata of acidic lakes typically contain low inorganic carbon concentrations (which equals CO_2 at low pH), potentially (co)limiting growth of *C. acidophila* (Tittel et al. 2005). In contrast, carbonate-rich groundwater inflow and bacterial respiration on the sediment surface result in high CO_2 concentrations in the deeper water strata (Tittel et al. 2003). In its natural environment, *C. acidophila* thus faces either a colimitation of P and CO_2 in the surface strata or is exposed to P-limiting but high CO_2 concentrations in the deeper strata. In the extreme habitat of acid lakes, *C. acidophila* is one of the few phytoplankton species that predominates and plays an important role in the food web (Kamjunke et al. 2004). Environmental conditions resulting in a change in its quality as a food source thus affects the efficiency of energy transfer to higher trophic levels in acidic habitats.

Laboratory studies on *C. acidophila* revealed that growth under ecologically relevant P-limiting and two different CO_2 concentrations resulted in P/CO_2-colimited growth (Spijkerman 2010). At the same steady state, P-limited growth rate, high CO_2-acclimated cells had higher maximum P-uptake rates (Spijkerman 2007) and lower photosynthetic rates (Spijkerman 2010) than low CO_2-acclimated cells, revealing that CO_2 aeration influenced P and CO_2 uptake rates although cells were at the same balanced growth rate. In these two studies, mixotrophic conditions (by addition of glucose) were additionally applied. In general, two mixotrophic strategies can be distinguished: osmo-mixotrophy where autotrophic growth is supplemented by dissolved organic compounds and phago-mixotrophy, where the ingestion of particles supplements growth. Here, we only consider the first as *Chlamydomonas* cannot ingest particles. Mixotrophically cultured *C. acidophila* had a similar maximum photosynthetic rate and cellular P quota (Q_p) as high CO_2 cells, but the algae were physiologically different because cellular growth was not colimited but only P-limited (Spijkerman 2010).

In studies on P-replete algae, different CO_2 and mixotrophic conditions resulted in changes in the fatty acid composition (Eichenberger 1976; Pronina et al. 1998; Boëchat et al. 2007; Wacker and Weithoff 2009). High PUFA content normally coincided with enhanced maximum photosynthetic rates (P_{max}), as thylakoid membranes are rich in PUFAs (e.g. Sato et al. 1996). Because *C. acidophila* cultured under low CO_2 conditions had a higher P_{max} than high CO_2 cells under P-limiting conditions (Spijkerman 2010), we expected low CO_2, P-limited cells to contain more PUFAs than high CO_2 cells.

In contrast to this hypothesis, high CO_2-acclimated cells of a neutral green alga, *Chlamydomonas reinhardtii,* had

the highest P_{max} but a lower percentage of the PUFAs 16:4(3,7,10,13) and 18:3(9,12,15) to total FA content compared to low CO_2-grown cells (Pronina et al. 1998). Similar differences in PUFA percentage can be found in high and low CO_2-grown cells of *C. reinhardtii* in a study by Sato when summing up his FA results from separate lipids (Sato 1989). Thus, high PUFA proportion does not always correlate with high P_{max}, which Pronina et al. (1998) explained by a positive relation between high PUFA demand as a response to the induction of a carbon concentrating mechanism that in their opinion requires high membrane fluidity.

Under mixotrophic, P-replete growth conditions, the PUFA concentration of *C. acidophila* was lower than under autotrophic conditions (Poerschmann et al. 2004). Indeed, photosynthetic rates under mixotrophic conditions were twofold lower than under autotrophic conditions (E. Spijkerman unpublished). As the P_{max} under a P-limitation in high CO_2 and mixotrophic conditions were similar (Spijkerman 2010), cells from those two treatments were expected to be equally low in PUFAs. As far as we know, for both CO_2 and mixotrophy, changes in the biochemical composition are unknown if an additional P-limitation is applied. In nature, and especially in very acidic waters where *C. acidophila* is found, adaptations to P and CO_2 limitation as well as mixotrophy are important for survival (Tittel et al. 2005; Spijkerman 2008a; Wolowski et al. 2008).

To study the changes in fatty acid composition in P/CO_2-(co)limiting conditions, we grew *C. acidophila* in P-limited semi-continuous cultures under high and low CO_2 and under photoautotrophic versus mixotrophic conditions. We specifically studied the interactions between the two growth-limiting factors.

Materials and methods

Cultures

Axenic cultures of *C. acidophila* Negoro (SAG Göttingen, strain no. 2045) were grown in semi-continuous cultures at 19.5 ± 1°C in Woods Hole medium (Nichols 1973), with no buffer, an inorganic P concentration of 1.6 µM and a pH adjusted to 2.7 (Spijkerman 2010). Mixotrophic growth was established by the addition of 1 mM glucose in the medium. This concentration will not pose osmolarity problems as concentrations inside the plant cell are typically hundredfold higher (Kehr et al. 1999). Dilution rates ranged from 0.1 to 0.6 day^{-1} in non-aerated cultures and from 0.2 to 1.0 day^{-1} in cultures aerated with 4.5% CO_2 in normal air (v/v) and in mixotrophic cultures (who were non-aerated). Incident light supply was approximately 200 µmol photons PAR m^{-2} s^{-1} with a light/dark period of 16/8 h. Daily dilution and harvesting were performed 4–5 h. after the onset of light. Average CO_2 concentrations in the CO_2-aerated cultures were measured as dissolved inorganic carbon using a carbon analyser (HighTOC+N, Elementar, Hanau, Germany) and were 0.33 (±0.05, $n = 20$) mM C. Non-aerated cultures contained a CO_2 concentration below the detection limit of the carbon analyser (<0.04 mM C) and were likely equal to equilibrium concentrations with the air (~0.02 mM C). The optical density of each culture was measured daily at 750 nm (UV1202, Shimadzu, Germany). After physiological acclimation was obtained (remaining at constant optical density after an exchange of 3–5 times the culture volume), dilution rate equalled growth rate (μ) and samples were taken for chemical analyses and fatty acid composition. Bacterial contamination in the cultures was checked on a regular basis under the microscope and was both low and similar in all treatments.

Cell numbers were determined on an automatic particle counter (CASY 1, Model TT, Schärfe, Reutlingen, Germany).

Chemical analyses

Cellular phosphorus quota expressed as mmolar P:molar C were determined by measuring the particulate P and C in the cultures. The particulate P concentration was determined on filtered culture suspension (0.2 µm Whatman nucleopore) heated to 100°C for 1 h with $K_2S_2O_8$ and 0.5 M H_2SO_4. Concentrations were determined spectrophotometrically using molybdate and ascorbic acid (Murphy and Riley 1962).

For particulate organic carbon analyses, culture samples were filtered on pre-combusted QF20 (Schleicher and Schuell) or GF/F filters (Whatman). The filters were analysed in the carbon analyser. Before measuring particulate organic carbon, filters were dried for 1 week at 30 or 50°C. Particulate organic carbon content can easily be recalculated in dry weight as earlier experiments have revealed that 50% of dry weight is carbon (Spijkerman 2007).

Fatty acid analysis

Samples for fatty acid determination were obtained by filtering whole cells, approximately 1 mg carbon on a glass fibre filter (GF/F, Whatman). Filters were stored at −25°C under nitrogen atmosphere in glass tubes with Teflon seal after adding 7 ml of dichlormethane-methanol (2:1 v/v) for the extraction of lipids (Sperfeld and Wacker 2009). Before further analysis, a defined concentration of tricosanoic acid methyl ester (23:0ME, Sigma-Aldrich) was added as internal standard. After extraction of lipids, identification and quantification of transesterified fatty acids was done by gas chromatography on an HP 6890N GC (Agilent

Technologies, Waldbronn, Germany) equipped with a flame ionization detector (FID) according to (Wacker and Martin-Creuzburg 2007) and verified earlier by GC/MS for this alga in which case also the positions of double bonds were identified (Poerschmann et al. 2004). All individual samples were measured at least twice. In a few cases, additionally heptadecanoic acid methylester (17:0ME) was used as internal standard. In the latter case, the standard and the FA methyl ester 16:3 eluted at the same time and therefore 16:3 had to be excluded from the analysis in these samples. All FAs were quantified by comparison with 23:0ME as internal standard and by using a multipoint standard calibration curve determined for each FA from mixtures of known composition (Sigma-Aldrich). FAs were normalized to the independently determined particulate organic carbon concentration of the sample.

Photosynthesis

Photosynthetic O_2 evolution rates were obtained in a light dispensation system (Topgallant LLC, Salt Lake City, USA). Cultures were concentrated by centrifugation (1,500*g*, 5 min) to a density of approximately 1.10^6 cells ml^{-1} and dark adapted for 30 min at 20°C. After that, oxygen evolution was measured over a range of eleven actinic light intensities (*I*, 0–1,500 µmol m^{-2} s^{-1}) using the light dispensation system over a 30-min period. From the concentrated culture suspension, samples for cell enumeration were taken. Rates of oxygen evolution were corrected for cell density (*Pr*) and fitted to the equation:

$$Pr = P_{\max}\left(1 - e^{-\alpha I/P_{\max}}\right) + I\beta + R_{\mathrm{d}}, \quad (1)$$

where $P_{\max}$ is the maximum gross oxygen production, R_{d} is the respiration rate in the dark, α is the initial slope of the photosynthesis–irradiance curve and β is the slope at high irradiances that describes photo-inhibition (Spijkerman 2010).

Statistical analysis

All statistical analyses were performed with the software R. Log10-transformed FA concentration was analysed in a three-way analysis of covariance (ANCOVA) using dilution rates or Q_{p} as continuous variable and both the carbon source (CO_2 or osmo-mixotrophic treatment) and the different fatty acid species as factors. After testing for assumptions of similar slopes (either $\mu \times$ treatment or $Q_{\mathrm{p}} \times$ treatment), multiple comparison tests following Zar (2010) were used as post hoc test to compare adjusted means over μ or Q_{p} between high or low CO_2 or osmo-mixotrophic treatments for individual fatty acids of interest. All data were used for correlations between either $P_{\max}$ or alpha and fatty acid concentrations of algae.

Results

We studied the changes in FA composition and content in *Chlamydomonas acidophila* as a result of P-limiting and additional high and low CO_2 and photoautotrophic versus mixotrophic conditions. The use of semi-continuous cultures resulted in more stringent P-limiting conditions at the lower growth rates (for residual P-concentrations see Spijkerman 2007). Nutrient enrichment experiments revealed that all cultures were P-limited and that the photoautotrophic high and low CO_2 cultures were additionally CO_2 limited (Spijkerman 2010). An overview of all data is provided in the electronic supplementary table S1: Here we present data of the most abundant FAs or those considered most important.

FA composition over growth rate

As hypothesized, there was both an effect of the growth rate and the carbon source on the fatty acid concentrations of *C. acidophila* (Table 1). An ANCOVA was run on the cellular concentration of all ten major FAs (16:0; 18:0; 18:1(9); 18:1(11); 20:1(11); 16:2(7,10); 16:3(7,10,13); 16:4(3,7,10,13); 18:2(9,12); 18:3(9,12,15)) and revealed that individual FA concentration changed with P-limitation (dilution rate), FA concentration differed between carbon sources (factor treatment), different FAs were present in different concentrations (factor FA), FA concentration varied differently for each FA with growth rate ($\mu \times$ FA; e.g. some increase, some remain the same) and different FAs vary differently with carbon source (treatment $\times$ FA; e.g. some are higher in low CO_2, and some lower) (Table 1). More detailed data are provided in S1.

More specifically on the effect of carbon source, the cellular concentration of 16:4(3,7,10,13) and 18:3(9,12,15) was higher in low CO_2 than in high CO_2 and mixotrophic cells (Fig. 1a, b), similar to results obtained with *Chlamydomonas reinhardtii* (Pronina et al. 1998). In *C. acidophila*, the difference in cellular concentrations was only significant at the two lowest steady state growth rates (i.e. only at severe P-limiting conditions, multiple comparison after Zar (2010); all $|q|_8 > 7.7$, $P < 0.01$; Fig. 1a, b). In contrast, 16:3(7,10,13) and 18:1(9) were lower in the low CO_2 than in high CO_2 and mixotrophic cells over all growth rates (multiple comparison following ANCOVA; all $|q|_{26} > 3.8$, $P < 0.05$; Fig. 1c, d). In addition, concentrations of the different PUFAs and MUFAs did not change with growth rate, except 18:2(9,12) (see S1) and 18:1(9)

Table 1 Results of the ANCOVA analysis of the ten major cellular FA concentrations of *C. acidophila* (in log [mg FA gC^{-1}]) with carbon treatment (either low or high CO_2 or mixotrophy with glucose) and in relation to steady state P-limited growth rate (μ, in day^{-1})

ANCOVA	*df*	Sum Sq	Mean Sq	*F* value	*P* value
Dilution rate (μ)	1	1.50	1.50	71.3	**<0.001**
Treatment ($\pm CO_2$, +Glc)	2	1.42	0.71	33.9	**<0.001**
FA	9	70.32	7.81	372.6	**<0.001**
μ × treatment	2	0.02	0.01	0.8	0.622
μ × FA	9	0.56	0.06	3.0	**<0.01**
Treatment × FA	18	1.23	0.07	3.3	**<0.001**
μ × treatment × FA	18	0.47	0.03	1.2	0.232
Residuals	240	5.03	0.02		

The significant values are given in bold

(Fig. 1d), which both decreased with increasing growth rate.

Both the concentrations of sums of SFA and MUFA changed with growth rate (see Fig. 2a for SFA) with higher concentrations present in cells with the lowest growth rates, i.e. in the most stringent P-limited cells. Over the whole range of growth rates tested, concentrations of SFA and MUFA were higher in the high CO_2 and mixotrophic cells than in low CO_2-grown cells. As a result of the changes in the concentrations of SFA and MUFA, but equal concentrations of PUFAs (most important PUFAs illustrated in Fig. 1a–c) with growth rate, the percentage of PUFA to total FA was higher in the low CO_2 cells, and this difference was largest at the two lowest growth rates tested (most stringent P-limited cells; Fig. 2b). The proportion of PUFAs in high CO_2 and mixotrophic cells was equal and increased with increasing growth rate from 40 to about 60% of total FA concentration. The low CO_2 cells always contained 60% PUFAs.

At the same steady state growth rates, i.e. the same P-limitation, different carbon sources resulted in a different cellular C and cellular P content (Fig. 3). Cells grown in high CO_2 or in mixotrophic conditions had a higher cellular C and lower cellular P content than those grown in low CO_2. To address for these physiological differences, we now present the FA composition of *C. acidophila* in relation to the Q_p to study the direct effect of P- and/or C-limitation.

FA composition over cellular P quota

Changes in FA composition with Q_p resulted in similar significant effects as with μ (compare Table 2 with Table 1), except for the following: the effect of treatment (CO_2/mixotrophy) was not significant ($P = 0.88$, Fig. 4a, c, d), and the effect of treatment × FA was only significant at the 5% level (Table 2). A closer look at the specific FAs in a post hoc test revealed that the significant effect of the interaction treatment × FA was solely a result of the fact that the concentration of the PUFA 18:3(9,12,15) was higher in the low CO_2 than in the mixotrophic cells ($P = 0.029$), and only marginally different from the high CO_2 cells ($P = 0.073$, Fig. 4b). Possibly, this effect is related to the presence of the most severe CO_2 (co)limitation in the low CO_2 cells and the absence of a CO_2 limitation in the mixotrophic cells (Spijkerman 2010). High CO_2 cells take an intermediate position as cells were also colimited by P and CO_2 in growth (Spijkerman 2010).

Overall, the FA composition changed with Q_p (Table 2) and the cellular concentration of most FAs decreased with increasing Q_p (Table 3; Fig. 4c, d). Only the concentration of 16:4(3,7,10,13) and 18:3(9,12,15) remained constant over Q_p (Table 3; Fig. 4a, b). The two PUFAs therefore responded more to carbon source (their concentration in low CO_2 cells was higher in the lower two growth rates tested; Fig. 1a, b) and less to P-limitation. The most pronounced pattern of FA change over Q_p was observed with the FAs 16:0, 18.0 and 18.1(9) (Table 3; Fig. 4d).

In general, a more stringent P-limitation (as indicated by the lower Q_p) in *C. acidophila* resulted in a higher total FA (SFA, MUFA and PUFA) concentration than less P-limited cells (regression analysis $t_{30} \leq -2.5$, $P < 0.05$; Fig. 5a). A similar pattern was observed for separate groups of SFA, MUFA and PUFA over Q_p, and resulted in a three- to fourfold higher concentrations of MUFAs and PUFAs than SFAs in all cases, although the quotient of unsaturated to saturated FA concentrations was the lowest in the most stringent P-limited algae.

Considering the total FA content over the cellular C and P content separately, a contrasting pattern is observed: Total FA content decreased with increasing cellular P content both ranging over a factor ten and total FA content increased over a twofold increasing cellular C content (Fig. 5b, c). A decreased cellular P content thus results in an enhanced FA accumulation, independent of cellular C status (Fig. 5; Table 3).

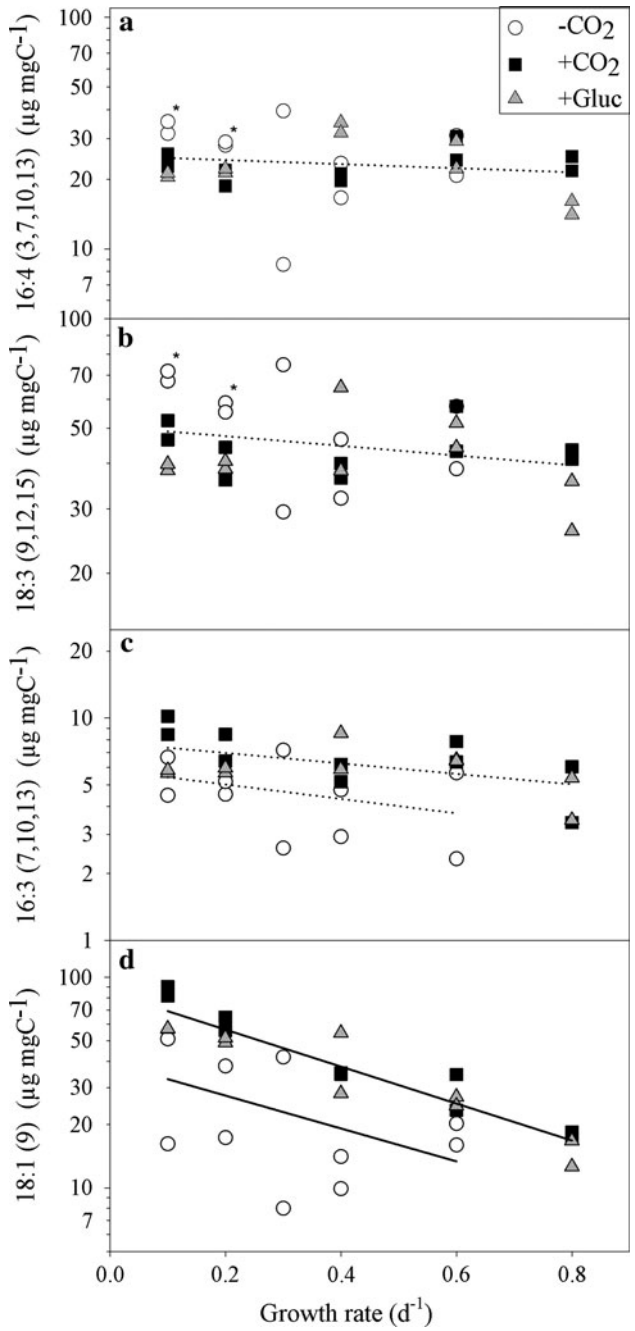

Fig. 1 Concentrations of **a** 16:4(3,7,10,13), **b** 18:3(9,12,15), **c** 16:3(7,10,13), and **d** 18:1(9) of *C. acidophila* in relation to steady state, P-limited growth rate. *Dashed lines* indicate non-significant changes over growth rate. In **a**, **b** the *asterisk* indicates a higher PUFA concentration in low CO_2 cells. *Lines* show linear regression through all points (**a**, **b**) or (after statistical grouping into homogenous data groups) through low CO_2 ($-CO_2$) and combining high CO_2 ($+CO_2$) and mixotrophic (+Gluc) treatments (**c**, **d**)

Relation to photosynthesis

The P_{max} negatively correlated to the total FA concentration (Fig. 6a; $r^2 = 0.46$, $P < 0.001$), thus the cells rich in

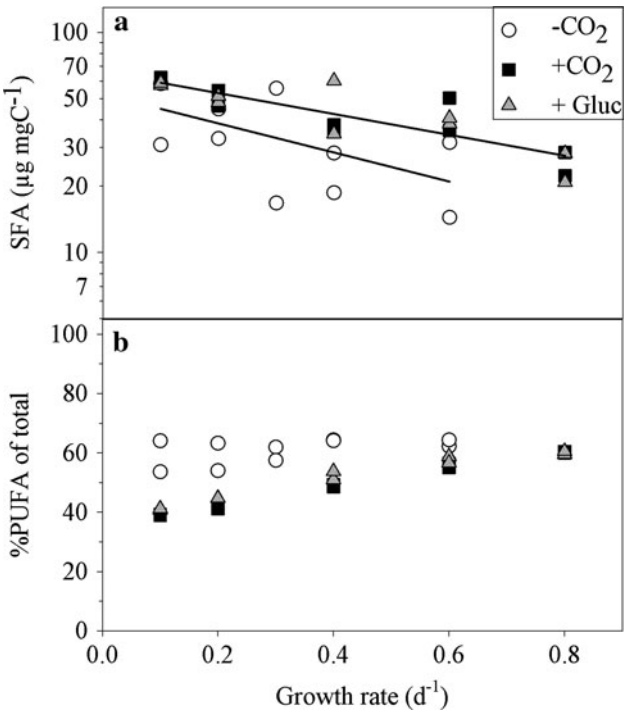

Fig. 2 Concentration of SFA (**a**), and percentage of PUFA to total FA content (**b**) of *C. acidophila* in relation to steady state, P-limited growth rate. *Lines* show linear regression through low CO_2 ($-CO_2$) and combining high CO_2 ($+CO_2$) and mixotrophic (+Gluc) treatments after statistical grouping into homogenous data groups

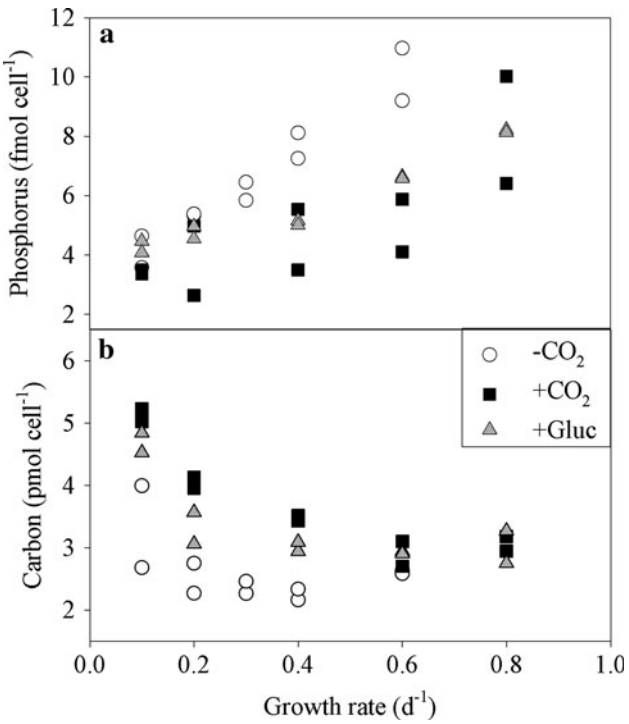

Fig. 3 Cellular P (**a**) and C (**b**) concentrations of *C. acidophila* in relation to steady state, P-limited growth rate. *Different symbols* denote low CO_2 ($-CO_2$), high CO_2 ($+CO_2$) or mixotrophic (+Gluc) growth conditions

Table 2 Results of the ANCOVA analysis of the cellular FA concentration of *C. acidophila* (in log [mg FA gC^{-1}]) with carbon treatment (either low or high CO_2 or mixotrophy with glucose) and in relation to cellular P quota (Q_p, in mmolP $molC^{-1}$)

ANCOVA	*df*	Sum Sq	Mean Sq	*F* value	*P* value
Cellular P quota (Q_p)	1	3.07	3.07	146.5	**<0.001**
Treatment (±CO_2, +Glc)	2	0.005	0.003	0.13	0.879
FA	9	70.32	7.81	373.1	**<0.001**
Q_p × treatment	2	0.02	0.01	0.5	0.590
Q_p × FA	9	0.96	0.11	5.1	**<0.001**
Treatment × FA	18	0.68	0.04	1.8	**<0.05**
Q_p × treatment × FA	18	0.46	0.03	1.2	0.236
Residuals	240	5.03	0.02		

The significant values are given in bold

FA had the lowest P_{max}. In contrast, the P_{max} increased with increasing percent PUFA concentration of the cells (Fig. 6b; $r^2 = 0.54$, $P < 0.001$). Consequently, a different result was obtained when the cellular PUFA concentration was expressed as a percentage of the total FA concentration rather than when the absolute PUFA concentration was considered. No correlation existed between the initial slope of the photosynthesis–irradiance curve (alpha) and total FA concentration or between the half saturation constant for CO_2 uptake and total FA concentration (not shown, but see data in S1). Similarly, no correlation was present between alpha and percent PUFA concentration, or for any of the individual fatty acids.

To further unravel which FAs contributed to the negative correlation with P_{max}, P_{max} was plotted against some individual FAs (Fig. 7). Against the expectation that P_{max} would positively correlate with cellular PUFA concentration (Sato et al. 1996; Pronina et al. 1998), P_{max} did not relate with the concentration of 16:4(3,7,10,13) and 18:3(9,12,15) when all data were combined (Fig. 7a, b; $r^2 < 0.029$, $P > 0.38$), although P_{max} did relate to the concentrations of these PUFAs when only the data from low CO_2 cells were analysed ($r^2 > 0.51$, $P < 0.05$; Fig. 7a, b). P_{max} negatively related with all the concentrations of 16:3(7,10,13) (Fig. 7c; $r^2 = 0.17$, $P < 0.05$) and 18:2(9,12) (Fig. 7f; $r^2 = 0.47$, $P < 0.001$). The most important FA contributing to the change in total FA was 18:1(9), as the cellular concentration varied between 8 and 90 µgC mgC^{-1} (Fig. 7d; $r^2 = 0.60$, $P < 0.001$). In addition, 16:0 decreased fivefold with increasing P_{max} (Fig. 7e; $r^2 = 0.62$, $P < 0.001$).

Discussion

In this study, we found a complex interplay between the different potentially limiting resources, phosphorus and carbon dioxide, which affected the fatty acid composition of the acidophilic green microalga *Chlamydomonas acidophila* (for generalized effects see Table 4).

The most pronounced effects in the changes in FA composition resulted from the P-limitation: stringent P-limited cells had a higher total fatty acid concentration and had a lower percentage of PUFAs. Under P-limitation, membrane lipid remodelling has often been observed in algae and plants: A portion of phospholipids are replaced by non-P galactolipids and sulfolipids (Andersson et al. 2003; Van Mooy et al. 2009). The non-P galactolipid DGDG is responsible for photosynthetic activity of photosystem II through the binding of extrinsic proteins required for stabilization of the oxygen-evolving complex, but not growth (Sakurai et al. 2007). DGDG may be required under P-limited conditions (Awai et al. 2007), as its percentage contribution to total lipids in cells of the cyanobacteria *Synechocystis* increased under P-deprivation. The main FA composition of DGDG in *Chlamydomonas reinhardtii* was 16:0, 18:1, 18:2 and 18:3 (Giroud et al. 1988), and a similar composition was reported for *C. acidophila* (Yamamoto et al. 1998). As consequently expected, the Q_p and P_{max} negatively correlated with the cellular content of 16:0, 18:1(9) and 18:2(9,12) (Table 3, Fig. 7d–f). Possibly, DGDG content was also more important under stringent P-limitation in *C. acidophila*, especially under low CO_2 conditions where the concentration of 18:3 also negatively correlated.

Another explanation for the changes in fatty acid composition in response to a P-limitation is an enhanced accumulation of lipids under P-deplete conditions. Inherent to the semi-continuous culturing system, more stringently P-limited cells have lower steady state growth rates. Consequently, lipid accumulation should increase with decreasing growth rates and Q_p. Accordingly, total FA content was highest in the most stringent P-limited cells, i.e. those at the lowest growth rate, with the lower Q_p (Fig. 5a). Similar to photosynthetic products produced under high light, P-deplete cells could accumulate

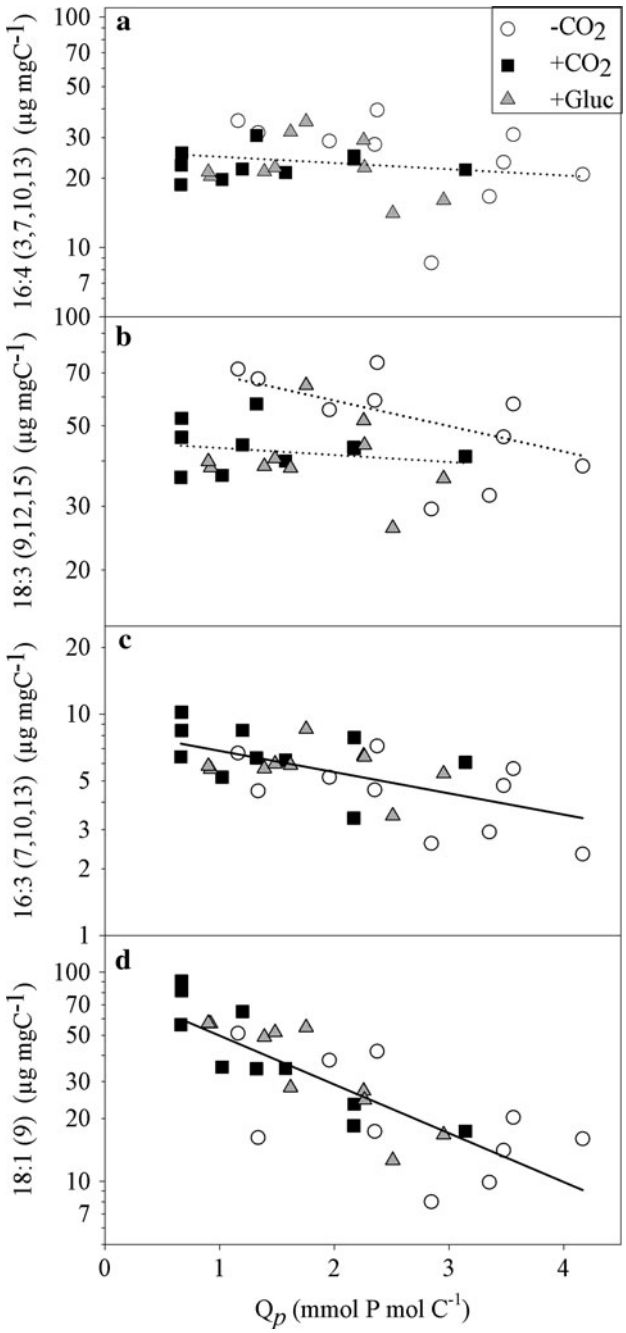

Fig. 4 Concentrations of **a** 16:4(3,7,10,13), **b** 18:3(9,12,15), **c** 16:3(7,10,13), and **d** 18:1(9) of *C. acidophila* in relation to Q_p. *Lines* show linear regression and *dashed lines* indicate non-significant changes over Q_p. *Lines* show linear regression through all points (**a**, **c**, **d**) or (after statistical grouping) through low CO_2 ($-CO_2$) and combining high CO_2 ($+CO_2$) and mixotrophic (+Gluc) treatments (**b**)

triacylglycerols (in addition to starch) that are rich in SFA and MUFA (Guschina and Harwood 2009). Indeed, SFA and MUFA concentrations in *C. acidophila* increased with decreasing growth rate and Q_p (Table 3; Fig. 2a), and especially high accumulations were observed in high CO_2 and mixotrophic cells. A negative correlation was observed between total FA concentration and Q_p (Fig. 5a) and has also been reported in the green alga *Scenedesmus quadricauda* (Ahlgren et al. 1998), the freshwater eustigmatophyte *Monodus subterraneus* (Khozin-Goldberg and Cohen 2006) and also in the cryptophyte *Rhodomonas salina* (Malzahn et al. 2007). As a result of the higher cellular C content in high CO_2 and mixotrophic cells, lipid accumulation under this most stringent P-limitation might be more pronounced than in low CO_2 cells.

In general, in green algae, a nutrient limitation coincides with lower PUFA content (Thompson 1996), which is only true for *C. acidophila*, if we consider the proportion of PUFA to total FA content as PUFA concentration itself was often constant over Q_p (Fig. 4a, b) or even increased with decreasing Q_p (Fig. 4c). Similar to *C. acidophila*, *n*-3-PUFA concentration increased in *Scenedesmus acutus* (now *S. obliquus*) or in a mixture of *Rhodomonas minuta*, *Scenedesmus acutus* and the cyanophyte *Synechococcus* sp. under P-limitation (Müller-Navarra 1995; Park et al. 2002). Most studies only report the percentage of PUFA to total FA content, which decreased with decreasing P availability in *C. acidophila* (see Fig. 2b). A similar observation was made in *Chlamydomonas moewusii* (Arisz et al. 2000), the bacillariophyte *Stephanodiscus hantzschii* var. *pusillus*, *Scenedesmus quadricauda* (Ahlgren et al. 1998), as well as in *C. reinhardtii* (Weers and Gulati 1997). As we show here, the effect of the change in PUFA proportion might well result from accumulations of other FAs while the PUFA concentration or cellular content might not change over Q_p.

The variation of the carbon source during P-limited growth in *C. acidophila* resulted in additional changes in its physiology and FA concentrations. As expected from the higher P_{max} in P-limited, low CO_2 than in P-limited high CO_2 cells (Spijkerman 2010), *C. acidophila* had a higher percentage of PUFAs in severely P-limited, low CO_2 than at high CO_2 and mixotrophic conditions (Fig. 2b). Consequently, our data support the positive relation between P_{max} and PUFA richness (Fig. 6b; Sato et al. 1996), which may overrule patterns related to external CO_2 and glucose concentration. Based on patterns related to CO_2 concentration, our results compare with several studies on P-replete algae and higher plants that report a higher proportion of PUFAs under low CO_2 conditions (Pronina et al. 1998; Riebesell et al. 2000; Ekman et al. 2007). Results for *C. reinhardtii* contrast with ours when comparing PUFA proportion and P_{max}, as the PUFA-rich, low CO_2 cells of *C. reinhardtii* had the lower P_{max} (Pronina et al. 1998). Most likely, this contrast results from a difference in acclimation strategy to low CO_2 conditions in both *Chlamydomonas* species. In *C. reinhardtii*, an acclimation to low CO_2 conditions results in a tenfold decrease in the half saturation constant for CO_2 uptake ($K_{m(CO2)}$; e.g.

Table 3 Results of the regression analysis of the different FA concentrations (expressed as log [mg FA gC^{-1}]) over Q_p (expressed as mmolP $molC^{-1}$) in *C. acidophila*

FA	*n*	R^2	*t* value	*P* value	Slope	SE
16:0	30	0.56	−5.98	**<0.001**	−0.130	±0.022
18:0	30	0.64	−7.02	**<0.001**	−0.157	±0.022
18:1(9)	30	0.64	−7.12	**<0.001**	−0.233	±0.033
18:1(11)	30	0.57	−6.12	**<0.001**	−0.131	±0.021
20:1(11)	30	0.15	−2.20	**<0.05**	−0.116	±0.053
16:2(7,10)	30	0.19	−2.59	**<0.05**	−0.064	±0.025
16:3(7,10,13)	30	0.37	−4.03	**<0.001**	−0.096	±0.024
16:4(3,7,10,13)	30	0.04	−1.05	0.30	−0.027	±0.026
18:2(9,12)	30	0.34	−3.83	**<0.001**	−0.101	±0.026
18:3(9,12,15)	30	0.02	−0.82	0.42	−0.018	±0.022

Significant values are given in bold

Amoroso et al. 1998) compared with high CO_2 conditions, whereas this was only threefold in *C. acidophila* (Spijkerman 2008b). Acclimation in the maximum uptake rate is pronounced in both *Chlamydomonas* species, which for *C. acidophila* reflects in a clear relation between PUFA or total FA concentration and P_{max} (Fig. 6), whereas PUFA or total FA concentration do not relate to the slope of the photosynthesis–irradiance curve (alpha) or with $K_{m(CO2)}$ (S1). In addition, there are more studies that contrast our results when focussing on CO_2 but support our results when relating PUFA richness to photosynthetic rates, e.g. the marine eustigmatophyte *Nannochloropsis* sp. had a lower PUFA content (as percentage of total FA content) in low CO_2 conditions that coincided with a lower biomass yield (suggesting a lower photosynthetic rate, Hu and Gao 2003). Possibly, one cannot compare a sum parameter between species, as different species (Dijkman and Kromkamp 2006) and even different strains of one species (Pronina et al. 1998) contain different PUFAs and sums of PUFAs cannot be connected with a specific physiological process. In addition, a large variety of acclimation strategies to changes in CO_2 concentration is present in different phytoplankton species (Tortell 2000). In conclusion, CO_2, glucose concentration or photosynthesis will not provide a full explanation of changes in FA composition comparing different species, although photosynthesis might better correlate with FA composition than the C-source.

More specifically at the level of single FA, we consider the PUFAs 16:4(3,7,10,13) and 18:3(9,12,15) that are characteristic of the chloroplast galactolipids (Thompson 1996) and the MUFA 18:1(9) that is a precursor FA for 18:3(9,12,15) in the chloroplast of *C. reinhardtii* (Sato et al. 1996). Consequently, the higher concentration of the PUFAs 16:4(3,7,10,13) and 18:3(9,12,15) (Fig. 1a, b) in combination with a lower accumulation of the precursor MUFA 18:1(9) (Fig. 1d) suggests a higher concentration of thylakoid membranes in severely P-limited, low CO_2 cells (at growth rates 0.1 and 0.2 day^{-1}). Low CO_2 cells of the chlorophyte *Chlorella kessleri* also had elevated proportions of 18:3(9,12,15) compared to high CO_2 cells (Sato et al. 2003). Sato et al. explained this as: decreased fatty acid synthesis caused lower growth rates and an enhanced desaturation of existing fatty acids. Comparing FA composition in different carbon treatments within the same growth rate as fixed by daily dilution rate excludes low growth as an explanation for higher PUFA proportions in low CO_2 cells of *C. acidophila* (Fig. 2b). The negative correlation of P_{max} with 18:1(9) under all conditions (Fig. 7d) supports the hypothesis that this FA is also in *C. acidophila* an important precursor for components of its thylakoid membranes. The cellular concentration of the PUFAs 16:4(3,7,10,13) and 18:3(9,12,15) did not positively relate to P_{max} (Fig. 7a, b) suggesting either compensatory regulation occurred in other organelles containing these two PUFAs, or the thylakoid membranes were partly inactive, i.e. some of them denied photosynthesis. This is most likely a result of fragmented chloroplasts caused by the accumulation of lipid bodies. The observed correlation between P_{max} and PUFA concentration in the low CO_2 cells suggests the presence of other regulatory mechanisms than in high CO_2 and mixotrophic cells.

High CO_2 and mixotrophic conditions resulted in a similar FA composition in *C. acidophila* (Figs. 1, 2), which supports previous results such as similar maximum photosynthetic and growth rates (Spijkerman 2007, 2010). Likely, both treatments can be considered as high CO_2 conditions, because intracellular CO_2 concentrations in mixotrophic conditions increase from internal glucose respiration (Villarejo et al. 1995). The lower P_{max} in mixotrophic, P-saturated cells of *C. acidophila*

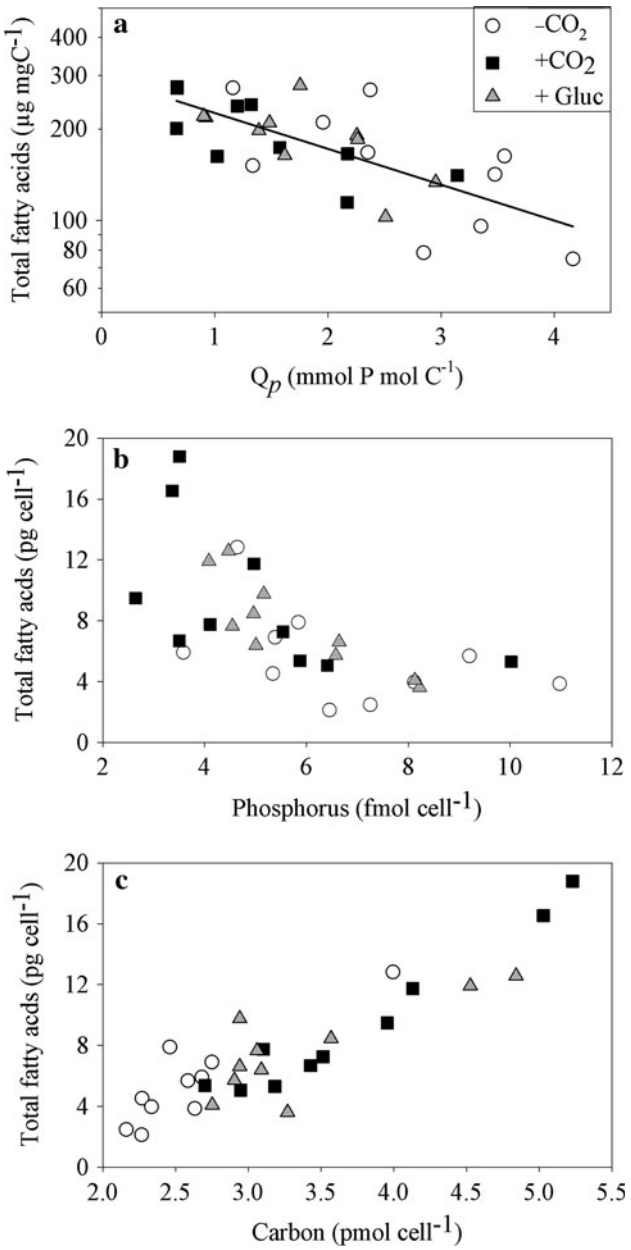

Fig. 5 Total FA concentration in relation to Q_p (**a**), cellular P (**b**) and C (**c**) content of *C. acidophila*. *Lines* show linear regression through all data points. *Different symbols* denote low CO_2 ($-CO_2$), high CO_2 ($+CO_2$) or mixotrophic (+Gluc) P-limited growth conditions

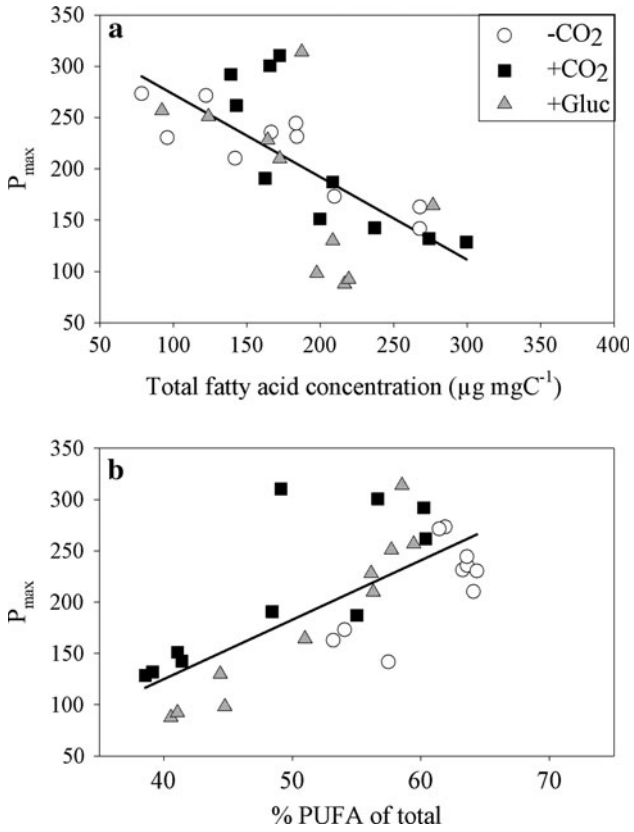

Fig. 6 Maximum photosynthetic rates (P_{max}, mmol O_2 10^{-12} cells h^{-1}) in relation to the total FA concentration (**a** in µg mgC^{-1}) and the percentage PUFA to total FA (**b**) of *C. acidophila*. *Different symbols* denote low CO_2 ($-CO_2$), high CO_2 ($+CO_2$) or mixotrophic (+Gluc) P-limited growth conditions

(E. Spijkerman, unpublished) coincide with a lower PUFA content than photo-autotrophic, low CO_2 cells (Poerschmann et al. 2004), or almost similar PUFA contents when only low glucose concentrations are provided (Wacker and Weithoff 2009). Besides a positive correlation between high PUFA proportion and high P_{max} (Fig. 6a), many other factors may influence PUFA content such as light (Guiheneuf et al. 2009) and temperature (Poerschmann et al. 2004) and the interaction between both (Piepho et al. 2011).

Ecological relevance and final conclusion

In extremely acidic lakes, the chlorophyte alga *C. acidophila* faces P-limiting conditions (Spijkerman 2008a) and possibly an additional CO_2-limitation (Tittel et al. 2005). In the low diversity ecosystem, the species is an important food item for herbivore predators (Kamjunke et al. 2004). The ecological relevance of FA composition of phytoplankton for herbivore predators such as daphnids was shown in field studies (Müller-Navarra et al. 2000; Wacker and Von Elert 2001). In addition, the FAs of *C. acidophila* were important both for rotifers' growth (Weithoff and Wacker 2007) and their competitive abilities (Hartwich et al. 2010). In one study, the concentration of PUFAs in three algal species explained more of the growth of *Daphnia* than the algal Q_p (Park et al. 2002).

To fully understand the cause of the reaction of each PUFA, a detailed analysis of the different lipid classes and their fatty acid composition might be useful. On the other hand, in food web studies and aquaculture, the fatty acid

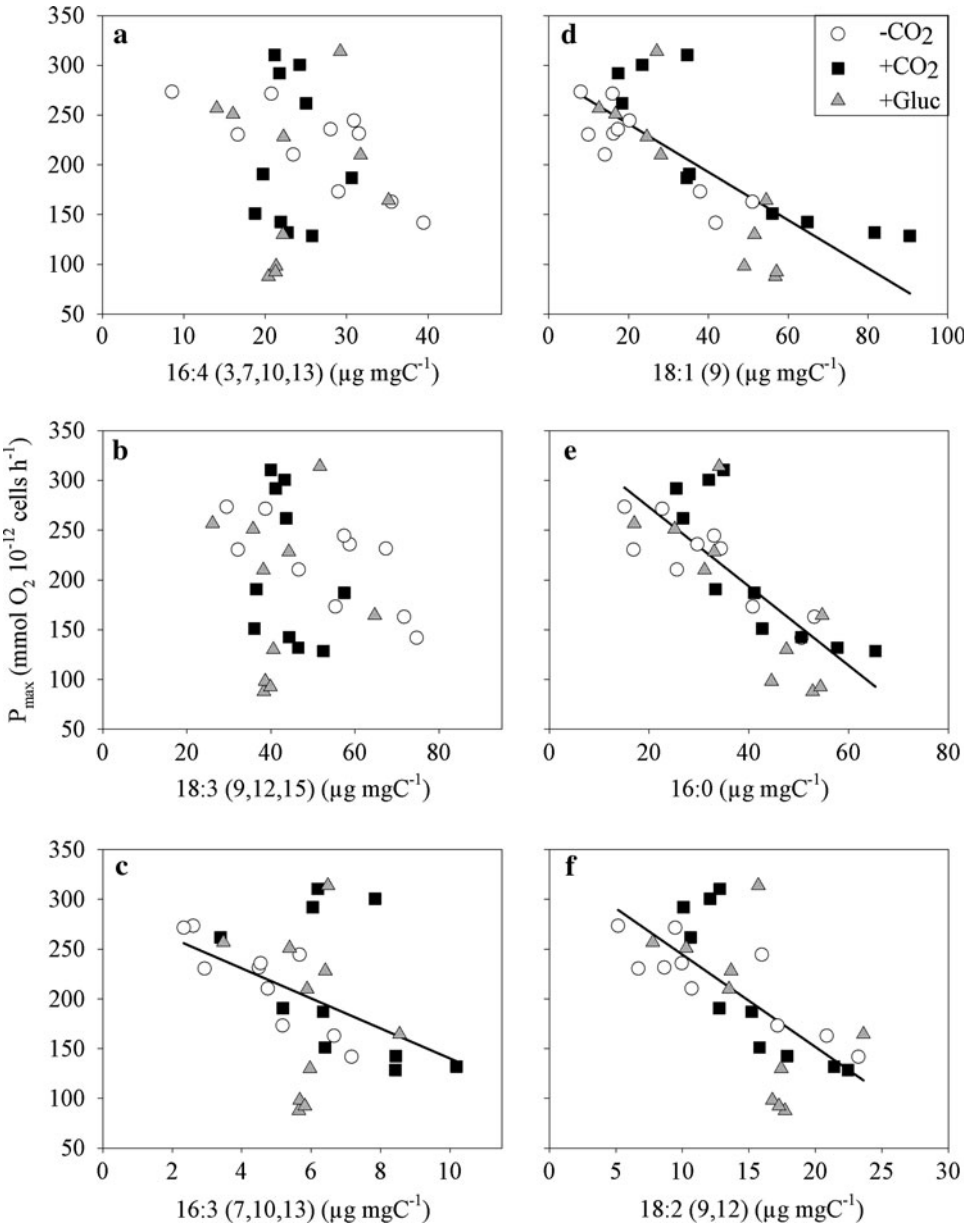

Fig. 7 Maximum photosynthetic rates (P_{max}, mmol O_2 10^{-12} cells h^{-1}) in relation to cellular concentrations of **a** 16:4(3,7,10,13), **b** 18:3(9,12,15), **c** 16:3(7,10,13), **d** 18:1(9), **e** 16:0, and **f** 18:2(9,12) of *C. acidophila*. *Different symbols* denote low CO_2 ($-CO_2$), high CO_2 ($+CO_2$) or mixotrophic (+Gluc) P-limited growth conditions

Table 4 Summary of relative differences in acclimation to stringent P-limited (−P) or less stringent P-limited (+P) conditions and high CO_2/mixotrophic (+C) or low CO_2 (−C) conditions in *C. acidophila*

Parameter	+P / –P	–P and +C / –P and –C
SFA	<	>
MUFA	<	>
PUFA	<	=
PUFA : total FA	>	<
P_{max}	>	<

composition of total lipids (as used in the present study) should be sufficient to assess the food quality of algae, as most herbivorous consumers ingest the whole algal cell. As a result of the relatively high concentrations of total FAs and PUFAs in P-limited algae, in terms of lipids (but not in terms of P) they could be considered high quality food for herbivores. Planktonic herbivores ingest whole cells and by engulfing a P-limited cell they receive a high concentration of total FAs that still consist of 40–60% of PUFAs. The present study also suggests that in addition to P-limitation, a CO_2-limitation of phytoplankton results in qualitatively rich food for herbivores with high FA and PUFA content.

Acknowledgments This work has been supported by the German research foundation (DFG, SP695/2 and SP695/4) to ES and (WA2445/4-1) to AW. We greatly acknowledge the technical assistance of Silvia Heim and Cathleen Friedrich.

References

Ahlgren G, Goedkoop W, Markensten H, Sonesten L, Boberg M (1997) Seasonal variations in food quality for pelagic and benthic invertebrates in Lake Erken—the role of fatty acids. Freshw Biol 38:555–570

Ahlgren G, Zeipel K, Gustafsson I-B (1998) Phosphorus limitation effects on the fatty acid content and nutritional quality of a green alga and a diatom. Verh int Ver Limnol 26:1659–1664

Amoroso G, Sültemeyer D, Thyssen C, Fock HP (1998) Uptake of HCO_3^- and CO_2 in cells and chloroplasts from the microalgae *Chlamydomonas reinhardtii* and *Dunaliella tertiolecta*. Plant Physiol 116:193–201

Andersson MX, Stridh MH, Larsson KE, Lijenberg C, Sandelius AS (2003) Phosphate-deficient oat replaces a major portion of the plasma membrane phospholipids with the galactolipid digalactosyldiacylglycerol. FEBS Lett 537:128–132

Arisz SA, van Himbergen JAJ, Musgrave A, van den Ende H, Munnik T (2000) Polar glycerolipids of *Chlamydomonas moewusii*. Phytochemistry 53:265–270

Arts MT, Kohler CC (2009) Health and condition in fish: the influence of lipids on membrane competency and immune response. In: Arts MT, Brett MT, Kainz MJ (eds) Lipids in aquatic ecosystems. Springer, New York, pp 237–255

Awai K, Watanabe H, Benning C, Nishida I (2007) Digalactosyldiacylglycerol is required for better photosynthetic growth of *Synechocystis* sp PCC6803 under phosphate limitation. Plant Cell Physiol 48:1517–1523

Boëchat IG, Weithoff G, Krüger A, Gücker B, Adrian R (2007) A biochemical explanation for the success of mixotrophy in the flagellate *Ochromonas* sp. Limnol Oceanogr 52:1624–1632

Dijkman NA, Kromkamp JC (2006) Phospholipid-derived fatty acids as chemotaxonomic markers for phytoplankton: application for inferring phytoplankton composition. Mar Ecol Prog Ser 324:113–125

Eichenberger W (1976) Lipids of *Chlamydomonas reinhardi* under different growth conditions. Phytochemistry 15:459–463

Ekman A, Bulow L, Stymne S (2007) Elevated atmospheric CO_2 concentration and diurnal cycle induce changes in lipid composition in *Arabidopsis thaliana*. New Phytol 174:591–599

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142

Giroud C, Gerber A, Eichenberger W (1988) Lipids of *Chlamydomonas reinhardtii*. Analysis of molecular species and intracellular site(s) of biosynthesis. Plant Cell Physiol 29:587–595

Guiheneuf F, Mimouni V, Ulmann L, Tremblin G (2009) Combined effects of irradiance level and carbon source on fatty acid and lipid class composition in the microalga *Pavlova lutheri* commonly used in mariculture. J Exp Mar Biol Ecol 369:136–143

Guschina IA, Harwood JL (2009) Algal lipids and effect of the environment on their biochemistry. In: Arts MT, Brett MT, Kainz MJ (eds) Lipids in aquatic ecosystems. Springer, New York, pp 1–24

Hartwich M, Wacker A, Weithoff G (2010) Changes in the competitive abilities of two rotifers feeding on mixotrophic flagellates. J Plankton Res 32:1727–1731

Hu HH, Gao KS (2003) Optimization of growth and fatty acid composition of a unicellular marine picoplankton. Nannochloropsis sp., with enriched carbon sources. Biotechnol Lett 25:421–425

Kamjunke N, Gaedke U, Tittel J, Weithoff G, Bell EM (2004) Strong vertical differences in the plankton composition of an extremely acidic lake. Arch Hydrobiol 161:289–306

Kehr J, Wagner C, Willmitzer L, Fisahn J (1999) Effect of modified carbon allocation on turgor, osmolality, sugar and potassium content, and membrane potential in the epidermis of transgenic potato (*Solanum tuberosum* L.) plants. J Exp Bot 50:565–571

Khozin-Goldberg I, Cohen Z (2006) The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte *Monodus subterraneus*. Phytochemistry 67:696–701

Lukas M, Sperfeld E, Wacker A (2011) Growth rate hypothesis does not apply across co-limiting conditions: cholesterol limitation affects phosphorus homeostasis of an aquatic herbivore. Funct Ecol. doi:10.1111/j.1365-2435.2011.01876.x

Malzahn AM, Aberle N, Clemmesen C (2007) Nutrient limitation of primary producers affects planktivorous fish condition. Limnol Oceanogr 52:2062–2071

Martin-Creuzburg D, Sperfeld E, Wacker A (2009) Colimitation of a freshwater herbivore by sterols and polyunsaturated fatty acids. Proc R Soc B Biol Sci 276:1805–1814

Müller-Navarra DC (1995) Biochemical versus mineral limitation in *Daphnia*. Limnol Oceanogr 40:1209–1214

Müller-Navarra DC, Brett MT, Liston AM, Goldman CR (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403:74–77

Murphy J, Riley JP (1962) A modified single solution method for determination of phosphate in natural waters. Anal Chim Acta 26:31–36

Nichols HW (1973) Growth media-freshwater. In: Stein JR (ed) Handbook of phycological methods: culture methods and growth measurements. Cambridge University Press, Cambridge, pp 7–24

Park S, Brett MT, Müller-Navarra DC, Goldman CR (2002) Essential fatty acid content and the phosphorus to carbon ratio in cultured algae as indicators of food quality for *Daphnia*. Freshw Biol 47:1377–1390

Piepho M, Arts MT, Wacker A (2011) Species-specific variation in fatty acid concentrations of four phytoplankton species: does phosphorus supply influence the effect of light intensity and temperature? J Phycol (accepted)

Poerschmann J, Spijkerman E, Langer U (2004) Fatty acid patterns in *Chlamydomonas* sp. as a marker for nutritional regimes and temperature under extremely acidic conditions. Microb Ecol 48:78–89

Pronina NA, Rogova NB, Furnadzhieva S, Klyachko-Gurvich GL (1998) Effect of CO_2 concentration on the fatty acid composition of lipids in *Chlamydomonas reinhardtii* cia-3, a mutant deficient in CO_2-concentrating mechanism. Russ J Plant Physiol 45:447–455

Riebesell U, Revill AT, Holdsworth DG, Volkman JK (2000) The effects of varying CO_2 concentration on lipid composition and carbon isotope fractionation in *Emiliania huxleyi*. Geochim Cosmochim Acta 64:4179–4192

Sakurai I, Mizusawa N, Wada H, Sato N (2007) Digalactosyldiacylglycerol is required for stabilization of the oxygen-evolving complex in photosystem II. Plant Physiol 145:1361–1370

Sato N (1989) Modulation of lipid and fatty acid content by carbon dioxide in *Chlamydomonas reinhardtii*. Plant Sci 61:17–21

Sato N, Sonoike K, Tsuzuki M, Kawaguchi A (1996) Photosynthetic characteristics of a mutant of *Chlamydomonas reinhardtii* impaired in fatty acid desaturation in chloroplasts. Biochim Biophys Acta Bioenerg 1274:112–118

Sato N, Tsuzuki M, Kawaguchi A (2003) Glycerolipid synthesis in *Chlorella kessleri* 11 h—II. Effect of the CO_2 concentration during growth. Biochim Biophys Acta Mol Cell Biol Lipids 1633:35–42

Sperfeld E, Wacker A (2009) Effects of temperature and dietary sterol availability on growth and cholesterol allocation of the aquatic keystone species *Daphnia*. J Exp Biol 212:3051–3059

Spijkerman E (2007) Phosphorus acquisition by *Chlamydomonas acidophila* under autotrophic and osmo-mixotrophic growth conditions. J Exp Bot 58:4195–4202

Spijkerman E (2008a) Phosphorus limitation of algae living in iron-rich, acidic lakes. Aquat Microb Ecol 53:201–210

Spijkerman E (2008b) What physiological acclimation supports increased growth at high CO_2 conditions? Physiol Plantarum 133:41–48

Spijkerman E (2010) High photosynthetic rates under a co-limitation for inorganic phosphorus and carbon dioxide. J Phycol 46:658–664

Sterner R (2008) On the phosphorus limitation paradigm for lakes. Int Rev Hydrobiol 93:433–445

Thompson GA (1996) Lipids and membrane function in green algae. Biochim Biophys Acta Lipids Lipid Metab 1302:17–45

Tittel J, Bissinger V, Zippel B, Gaedke U, Bell E, Lorke A, Kamjunke N (2003) Mixotrophs combine resource use to outcompete specialists: Implications for aquatic food webs. Proc Natl Acad Sci USA 100:12776–12781

Tittel J, Bissinger V, Gaedke U, Kamjunke N (2005) Inorganic carbon limitation and mixotrophic growth in *Chlamydomonas* from an acidic mining lake. Protist 156:63–75

Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish Sci 11:107–184

Tortell PD (2000) Evolutionary and ecological perspectives on carbon acquisition in phytoplankton. Limnol Oceanogr 45:744–750

Van Mooy BAS, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, Koblizek M, Lomas MW, Mincer TJ, Moore LR, Moutin T, Rappe MS, Webb EA (2009) Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. Nature 458:69–72

Villar-Argaiz M, Medina-Sanchez JM, Bullejos FJ, Delgado-Molina JA, Perez OR, Navarro JC, Carrillo P (2009) UV radiation and phosphorus interact to influence the biochemical composition of phytoplankton. Freshw Biol 54:1233–1245

Villarejo A, Orus MI, Martinez F (1995) Coordination of photosynthetic and respiratory metabolism in *Chlorella vulgaris* UAM-101 in the light. Physiol Plantarum 94:680–686

Wacker A, Martin-Creuzburg D (2007) Allocation of essential lipids in *Daphnia magna* during exposure to poor food quality. Funct Ecol 21:738–747

Wacker A, Von Elert E (2001) Polyunsaturated fatty acids: evidence for non-substitutable biochemical resources in *Daphnia galeata*. Ecology 82:2507–2520

Wacker A, Weithoff G (2009) Carbon assimilation mode in mixotrophs and the fatty acid composition of their rotifer consumers. Freshw Biol 54:2189–2199

Weers PMM, Gulati RD (1997) Growth and reproduction of *Daphnia galeata* in response to changes in fatty acids, phosphorus, and nitrogen in *Chlamydomonas reinhardtii*. Limnol Oceanogr 42:1584–1589

Weithoff G, Wacker A (2007) The mode of nutrition of mixotrophic flagellates determines the food quality for their consumers. Funct Ecol 21:1092–1098

Wolowski K, Turnau K, Henriques FS (2008) The algal flora of an extremely acidic, metal-rich drainage pond of Sao Domingos pyrite mine (Portugal). Cryptogamie Algol 29:313–324

Yamamoto Y, Tatsuzawa H, Wada M (1998) Effect of environmental conditions on the composition of lipids and fatty acids in *Chlamydomonas* isolated from an acidic lake. Verh Int Ver Theor Angew Limnol 26:1788–1790

Zar JH (2010) Biostatistical analysis, 5th edn. Prentice Hall/Pearson Education, London