



Acyl lipid composition variation related to culture age and nitrogen concentration in continuous culture of the microalga *Phaeodactylum tricornutum*

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Abstract

The influence of culture age and nitrogen concentration on the distribution of fatty acids among the different acyl lipid classes has been studied in continuous cultures of the microalga *Phaeodactylum tricornutum*. The culture age was tested in the range of 1.15–7 days, controlled by adjusting the dilution rate of fresh medium supplied. The effect of nitrogen concentration was tested from saturating conditions to starvation by modifying nitrate concentration in the fresh medium. Culture age had almost no influence on the fatty acid content; 16:0, 16:3 and 20:5 increased moderately wherein the level of 16:1 decreased when the culture age decreased. Culture age had no effect on the total fatty acid content that remained around 11% of dry weight. Conversely, culture age had a greater impact on lipid classes, producing changes in amounts of triacylglycerols (TAG) which ranged between 43% and 69%, and galactolipids (GLs) that oscillated between 20% and 40%. In general, the content of polar lipids of the biomass decreased with culture age. The other factor assayed, nitrogen content, affected the fatty acid profile. Saturated and monounsaturated fatty acids accumulated when the nitrogen concentration was decreased. The experiments regarding the effect of nitrogen concentration on lipid species were carried out with cells of an average age of 3.5 days. A decrease of the nitrogen concentration caused the GL fraction to decrease from 21 to 12%. Conversely, both neutral lipids (NLs) and phospholipids (PLs) increased from about 73 to 79% and from 6 to 8%, respectively. In these experiments, TAG was the lipid class with the highest increase, from 69 to 75%. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Phaeodactylum tricornutum*; Bacillariophyceae; Acyl lipid composition; Polyunsaturated fatty acids; Continuous culture; Cell age; Nitrogen concentration

1. Introduction

In a previous paper, we reported on the dramatic changes produced in the lipid profile of the diatom *Phaeodactylum tricornutum* Bohlin (López Alonso et al., 1998) depending on whether it was cultured indoor

(stationary phase; batch culture) or outdoor (exponential phase; continuous culture). These changes were primarily concerned with neutral lipids (NLs) and glycolipids (GLs) that evolved in mutually opposite directions: old cells were richer in NLs and poorer in GLs than younger cells. However, the indoor and outdoor cultures were from different devices and modes of culture, thus the specific causes of the above differences in lipid profiles were difficult to assign.

Wide differences in fatty acid composition related with culture conditions are commonly observed in

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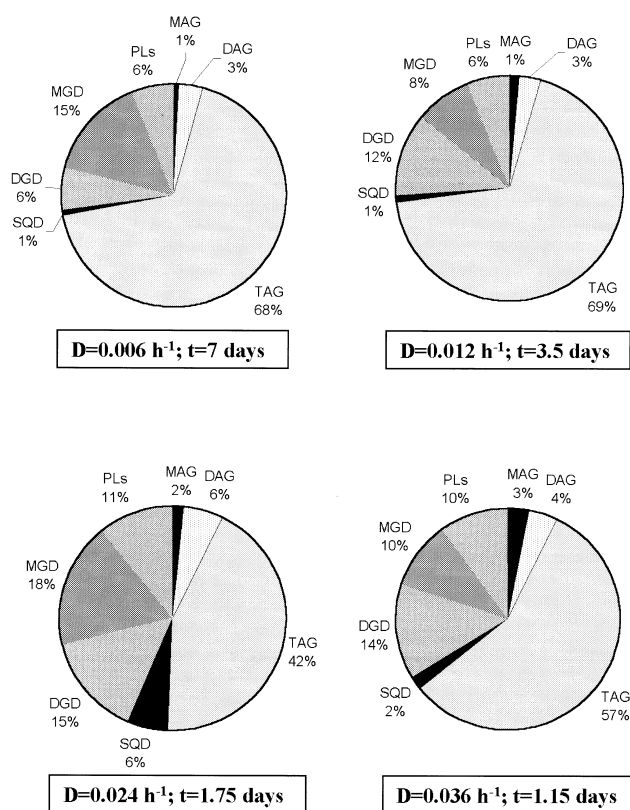


Fig. 1. Effect of the average residence time on the relative proportion of the different lipid classes.

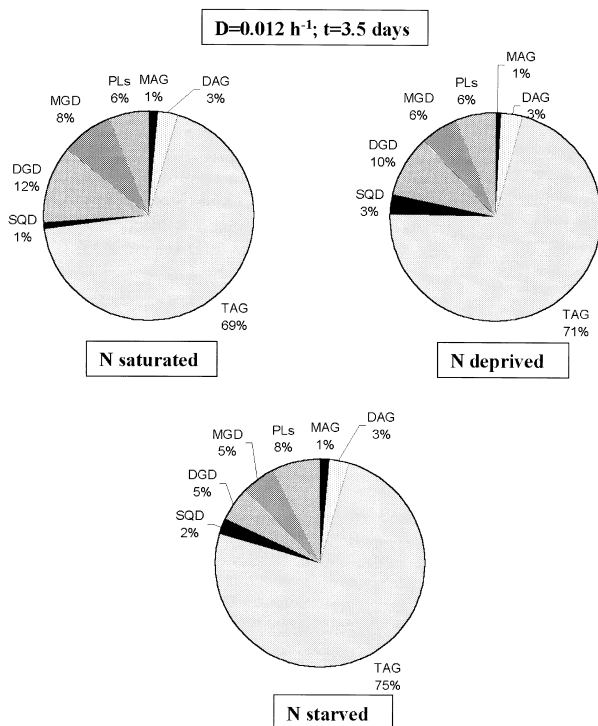


Fig. 2. Effect of nitrogen deprivation on the relative proportion of the different lipid classes at low dilution rate ($D = 0.012 \text{ h}^{-1}$).

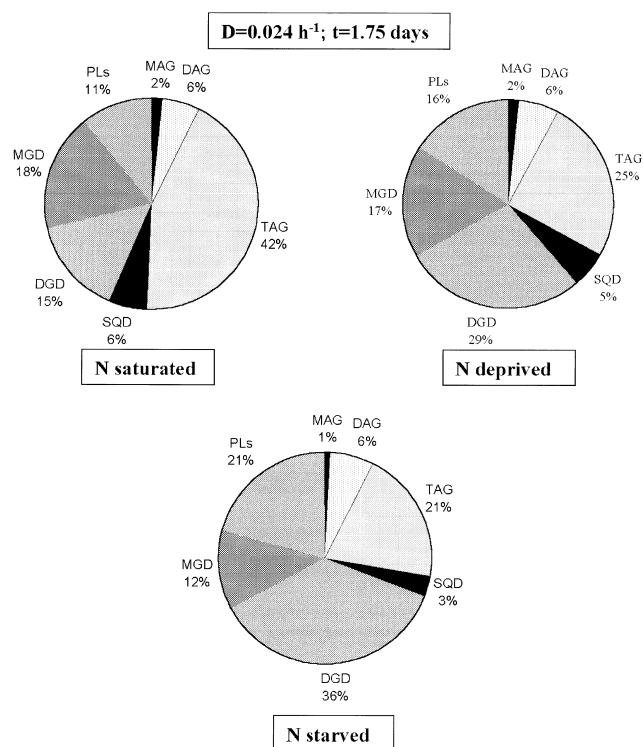


Fig. 3. Effect of nitrogen deprivation on the relative proportion of the different lipid classes at high dilution rate ($D = 0.024 \text{ h}^{-1}$).

microalgae (Pohl and Zurheide, 1979; Álvarez Cobelas, 1989). Increase in lipid content is usually associated with culture ageing (i.e., stationary phase) (Piorreck and Pohl, 1984; Siron et al., 1989; Hodgson et al., 1991; Ahlgren et al., 1992; Dunstan et al., 1993; Lombardi and Wangersky, 1995; Bell and Pond, 1996; López Alonso et al., 1998). On the other hand, nitrogen stress (Pohl and Zurheide, 1979; Shifrin and Chisholm, 1980; Piorreck and Pohl, 1984; Parrish and Wangersky, 1987; Álvarez Cobelas, 1989), and nutrient limitations in general (Reitan et al., 1994) have also been positively associated with increase in lipid abundance, although the response was species-specific (Shifrin and Chisholm, 1980; Lombardi and Wangersky, 1995). Moreover, ageing effects may be indistinguishable from nutrient limitation effects because ageing generally involves depletion of some nutrient (Álvarez Cobelas, 1989) at least in batch culture which was the usual culture system in most earlier works. Also, most of these studies on microalgal lipids have focussed only on total lipids or fatty acids. For a better understanding of the changes in amounts of the individual lipid classes need to be analysed.

This study examines the effects of culture age and nitrogen availability on the lipid composition of *P. tri-cornutum* in steady-state continuous cultures where the effects of age and nitrogen limitation are not con-

founded. Four different steady-states were obtained corresponding to four different dilution rates ($D_1 = 0.006 \text{ h}^{-1}$, $D_2 = 0.012 \text{ h}^{-1}$, $D_3 = 0.024 \text{ h}^{-1}$, and $D_4 = 0.036 \text{ h}^{-1}$). The dilution rate (D) is the ratio of the volumetric feed rate (fresh medium) to the total reactor volume (5 l). Therefore, D represents the volume fraction replaced per unit time while the inverse dilution rate ($1/D$) is the time it takes for the total reactor volume to be replaced and thus is the average time a cell spends within the reactor. At the lowest dilution rate tested ($D_1 = 0.006 \text{ h}^{-1}$) culture age was 7 days, and at the highest ($D_4 = 0.036 \text{ h}^{-1}$) culture age was 1.15 days. A broad variety of culture states was obtained under these conditions because 7 days old biomass corresponds roughly to stationary phase cultures while 1.15 days (less than 28 h) are well representative of young cells. Because in a continuous culture, steady-state is only attained when the specific growth

rate μ equals the dilution rate (i.e. $\mu = D$), the growth rate of the cells is also readily known in each experiment. At dilution rates D_2 and D_3 , three nitrogen concentrations were tested: 2 mM (N-starved), 5 mM (N-deprived), and 10 mM (N-saturated).

2. Experimental

The microalga used was *P. tricornutum* UTEX640. Cultures were grown in a cylindrical ($d = 0.17 \text{ m}$) 5-l computer-controlled fermentor (New Brunswick Scientific Bioflo III). Culture medium was prepared in seawater that had been filtered through $0.22 \text{ }\mu\text{m}$ -pore Millipore filters and then autoclaved at 120°C for 1 h. The medium contained sterile seawater enriched with 20 ml^{-1} of a sterile Ukeles stock solution modified as suggested by Molina

Table 1

Lipid and fatty acid composition of *P. tricornutum* cultured at dilution rate 1 ($D = 0.006 \text{ h}^{-1}$)^a

	Lipid ^b (% of fatty acids)												Biomass
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	PLX1	PLX2	
14:0	5.8 (1.4)	10.1 (3.5)	11.0 (1.0)	5.4 (1.8)	4.8 (0.0)	2.5 (0.6)	0.0 (0.0)	1.8 (0.2)	9.8 (6.3)	0.0 (0.0)	0.0 (0.0)	5.6 (1.1)	8.4 (0.1)
16:0	11.7 (1.0)	7.8 (0.3)	5.3 (0.2)	30.6 (8.1)	10.5 (2.0)	6.4 (0.1)	14.0 (0.0)	4.9 (2.5)	16.6 (8.4)	20.1 (1.3)	29.4 (14.4)	19.2 (1.6)	9.4 (0.1)
16:1	24.4 (0.8)	9.1 (1.4)	25.0 (0.6)	43.5 (13.1)	33.4 (1.9)	27.9 (0.3)	31.2 (0.2)	27.3 (1.0)	32.3 (1.0)	33.5 (7.2)	21.9 (21.9)	14.2 (0.1)	25.8 (0.2)
16:2n-4	5.7 (1.3)	2.0 (0.3)	3.4 (0.2)	0.0 (0.0)	5.9 (4.2)	9.1 (0.2)	0.0 (0.0)	2.0 (0.5)	1.9 (1.9)	18.2 (18.2)	0.0 (0.0)	3.2 (3.2)	3.8 (0.0)
16:3n-4	14.1 (1.3)	7.9 (0.1)	4.6 (0.1)	2.2 (2.2)	18.3 (2.0)	26.7 (0.1)	0.0 (0.0)	0.5 (0.5)	6.3 (6.3)	0.0 (0.0)	0.0 (0.0)	4.3 (0.5)	8.2 (0.2)
16:4n-1	0.0 (0.0)	0.0 (0.0)	0.2 (0.0)	0.0 (0.0)	0.2 (0.2)	0.7 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (0.0)
18:1n-9	0.0 (0.0)	0.3 (0.3)	0.5 (0.0)	0.0 (0.0)	1.2 (0.6)	1.5 (0.2)	6.2 (1.3)	4.7 (0.4)	1.5 (1.5)	0.0 (0.0)	0.0 (0.0)	6.3 (1.5)	0.9 (0.1)
18:1n-7	0.0 (0.0)	0.0 (0.0)	0.3 (0.0)	0.0 (0.0)	1.0 (0.4)	0.5 (0.1)	0.8 (0.9)	1.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.5 (0.0)
18:2n-6	2.9 (0.0)	1.0 (0.3)	1.3 (0.0)	6.2 (1.3)	4.5 (2.7)	3.3 (0.1)	20.4 (3.0)	20.8 (2.4)	12.2 (1.0)	9.8 (9.8)	4.6 (4.6)	10.8 (3.5)	2.6 (0.0)
20:4n-6	0.0 (0.0)	0.0 (0.0)	1.0 (0.0)	0.0 (0.0)	0.5 (0.1)	0.4 (0.0)	0.9 (0.9)	2.6 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.9 (0.0)
20:5n-3	21.2 (1.5)	8.3 (1.8)	35.5 (0.3)	1.8 (1.8)	13.2 (5.2)	8.8 (0.1)	8.0 (1.1)	8.8 (0.2)	7.5 (7.5)	18.3 (0.1)	5.7 (5.7)	8.2 (1.6)	25.7 (0.2)
22:6n-3	2.7 (0.1)	0.4 (0.4)	0.6 (0.1)	1.8 (1.8)	2.1 (1.2)	1.7 (0.1)	7.8 (1.0)	10.9 (1.0)	3.0 (3.0)	0.0 (0.0)	0.0 (0.0)	2.0 (2.0)	1.8 (0.1)
USCFAs ^c	7.1 (1.0)	51.7 (7.9)	8.1 (2.2)	0.0 (0.0)	0.0 (0.0)	1.9 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.9 (4.9)	5.3 (2.5)	6.3 (0.1)
Others	4.3	1.3	3.2	8.4	4.4	8.7	10.8	14.6	8.8	0.0	33.5	20.9	5.4

^a Values are averages of three independent measurements. Standard errors are shown in parentheses.

^b MAG, DAG and TAG stated for mono-, di-, and triacylglycerols, respectively; SQD, DGD, and MGD stated for sulfoquinovosyl-, digalactosyl-, and monogalactosyldiacylglycerols, respectively; PI, PC, PG, and PE stated for phosphatidylinositol, -choline, -glycerol, and -ethanolamine, respectively; PLX are unknown phospholipids.

^c USCFAs = unknown short-chain fatty acids. For additional details see López Alonso et al. (1998).

Grima et al. (1994). Nitrogen concentration was changed by decreasing the quantity of sodium nitrate used to prepare the stock solution to one-half (5 mM) and one-fifth (2 mM) of the original content. While the original composition was saturating for nitrogen in the culture conditions used, the one-half reduced concentration was intended to cause a slight deprivation and the one-fifth reduced concentration was intended to produce a severe nitrogen limitation.

The culture was illuminated by six cool-white fluorescent lamps (Osram Dulux EL, 20 W) regularly spaced around the culture vessel. This produced a scalar irradiance of $1200 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (PAR) throughout the culture. Irradiance was measured with a Quantum Scalar Irradiance Meter QSL-100 Biospherical Instruments Laboratory (San Diego, CA). Temperature was kept at 20°C. The air supply was sterilized by filtration through 0.22 μm Millipore filters at a rate of $1.5 \text{ l}\cdot\text{min}^{-1}$. Agitation speed was set at 150 rpm and

the pH was maintained constant at 8 by on-demand injection of pure CO_2 mixed with the air supply.

The experiments were done in continuous mode. For this, the cultures were started in batch mode with an initial addition of *P. tricornutum* inoculum to 5 l medium contained in the photobioreactor to give a starting biomass concentration of around $50 \text{ mg}\cdot\text{l}^{-1}$. The cultures were then kept in batch mode until a biomass concentration of 500–700 $\text{mg}\cdot\text{l}^{-1}$ was attained and then the continuous operation was started by adding continuously the fresh medium. The dilution rate, D , for each experiment was set using a programmable peristaltic pump. The cultures were then developed until a steady-state was attained. At each steady-state the runtime was at least 4 days. The biomass was then harvested by centrifugation.

Four different steady-states were obtained at four different dilution rates ($D_1 = 0.006 \text{ h}^{-1}$, $D_2 = 0.012 \text{ h}^{-1}$, $D_3 = 0.024 \text{ h}^{-1}$, and $D_4 = 0.036 \text{ h}^{-1}$). Average

Table 2

Lipid and fatty acid composition of *P. tricornutum* cultured at dilution rate 2 ($D = 0.012 \text{ h}^{-1}$)^a

	Lipid ^b (% of fatty acids)												Biomass
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	PLX1	PLX2	
14:0	2.4 (1.2)	11.5 (1.5)	6.1 (3.3)	4.1 (4.1)	0.6 (0.6)	3.5 (3.5)	1.8 (1.8)	1.0 (1.0)	2.5 (2.5)	0.0 (0.0)	0.0 (0.0)	3.0 (3.0)	7.8 (0.1)
16:0	11.8 (1.2)	8.9 (1.3)	6.5 (0.1)	27.1 (0.3)	9.8 (1.1)	11.8 (1.3)	11.4 (1.0)	11.8 (2.8)	12.1 (0.9)	14.5 (3.4)	17.0 (0.2)	17.7 (2.2)	10.5 (0.1)
16:1	17.0 (3.7)	12.2 (0.7)	20.7 (1.0)	41.7 (0.9)	20.3 (0.1)	21.2 (1.0)	19.3 (0.8)	24.6 (1.5)	21.3 (1.6)	26.7 (4.0)	21.6 (1.8)	9.7 (1.7)	22.7 (0.2)
16:2n-4	5.6 (0.9)	3.8 (0.0)	4.1 (0.2)	4.7 (0.4)	11.7 (0.5)	6.4 (0.7)	2.5 (0.2)	2.8 (0.2)	3.1 (3.1)	0.0 (0.0)	0.0 (0.0)	0.6 (0.6)	4.8 (0.1)
16:3n-4	14.9 (0.2)	11.6 (0.2)	6.8 (0.2)	4.6 (0.4)	40.2 (2.6)	15.8 (2.7)	1.0 (1.0)	1.4 (0.0)	12.4 (3.4)	14.8 (11.1)	11.4 (2.9)	2.5 (0.3)	9.9 (0.7)
16:4n-1	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)	0.6 (0.0)	0.2 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.0)
18:1n-9	0.8 (0.1)	0.4 (0.4)	1.9 (1.3)	0.8 (0.8)	0.6 (0.2)	2.1 (0.5)	5.3 (0.9)	5.0 (0.2)	1.5 (1.5)	1.8 (1.8)	0.0 (0.0)	8.9 (2.9)	1.3 (0.3)
18:1n-7	0.0 (0.0)	0.0 (0.0)	0.3 (0.0)	0.0 (0.0)	0.5 (0.0)	0.6 (0.0)	1.7 (0.2)	1.4 (0.1)	1.5 (1.5)	2.0 (2.0)	0.0 (0.0)	0.0 (0.0)	0.6 (0.0)
18:2n-6	1.8 (0.1)	1.5 (0.3)	1.4 (0.0)	3.5 (0.3)	1.7 (0.1)	4.0 (0.3)	21.6 (2.1)	20.1 (2.3)	11.7 (1.0)	6.7 (6.7)	11.9 (4.9)	11.3 (1.5)	2.6 (0.0)
20:4n-6	0.0 (0.0)	0.0 (0.0)	0.5 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	1.0 (0.0)	0.6 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.4 (0.1)
20:5n-3	18.1 (7.4)	13.4 (2.6)	32.3 (2.4)	2.8 (0.8)	5.3 (0.1)	19.0 (3.3)	8.4 (1.3)	7.4 (1.5)	11.5 (0.2)	20.0 (5.7)	13.6 (0.7)	6.1 (0.2)	25.3 (0.1)
22:6n-3	2.1 (0.1)	1.4 (0.3)	0.5 (0.0)	0.0 (0.0)	1.4 (0.0)	1.6 (0.1)	11.6 (1.2)	11.2 (3.6)	8.2 (1.0)	4.5 (4.5)	6.1 (6.1)	3.2 (0.8)	1.7 (0.0)
USCFAs ^c	16.0 (8.1)	33.2 (3.2)	11.9 (3.7)	2.2 (2.2)	1.1 (1.1)	5.1 (0.1)	0.0 (0.0)	1.3 (1.3)	4.8 (4.8)	3.3 (3.3)	4.3 (4.3)	3.3 (3.3)	6.8 (0.4)
Others	9.6	2.1	7.0	8.6	6.2	8.6	14.4	11.6	9.3	5.8	14.2	33.6	5.5

^a Values are averages of three independent measurements. Standard errors are shown in parentheses.

^b MAG, DAG and TAG stated for mono-, di-, and triacylglycerols, respectively; SQD, DGD, and MGD stated for sulfoquinovosyl-, digalactosyl-, and monogalactosyldiacylglycerols, respectively; PI, PC, PG, and PE stated for phosphatidylinositol, -choline, -glycerol, and -ethanolamine, respectively; PLX are unknown phospholipids.

^c USCFAs = unknown short-chain fatty acids. For additional details see López Alonso et al. (1998).

cell ages were 7, 3.50, 1.75, and 1.15 days at D_1 , D_2 , D_3 , and D_4 , respectively. At the two near-optimal dilution rates (D_2 and D_3 , experiments were carried out also at one-half and one-fifth of the standard nitrogen concentration. Overall, eight different steady-states (experiments) were attained and analysed.

Biomass concentration in the culture was estimated by optical density (Molina Grima et al., 1993) and checked under steady-state conditions by dry weight determinations. Fatty acid and lipid class analyses were done as described elsewhere (López Alonso et al., 1998). Values for 16:1 probably included two isomers (16:1n7 and 16:1n13*trans*) in the case of the plastidial lipid class PG (López Alonso et al., 1998).

For each steady-state, three samples of biomass were extracted and analysed separately. The data were averaged.

3. Results and discussion

The data presented were obtained using microalgal biomass harvested from steady-state continuous cultures. The data refer exclusively to acyl lipids and the discussion is restricted to the main fatty acids (those that constitute 5% or more of total fatty acids) that can be reliably estimated. These fatty acids were 14:0, 16:0, 16:1, 16:2, 16:3, and 20:5. The corresponding result compositions are reported in Tables 1–8 for various conditions.

3.1. Changes related with culture age

3.1.1. Fatty acid composition

Only slight changes appeared in the fatty acid composition of *P. tricornutum* biomass when the culture

Table 3
Lipid and fatty acid composition of *P. tricornutum* cultured at dilution rate 3 ($D = 0.024 \text{ h}^{-1}$)^a

	Lipid ^b (% of fatty acids)												Biomass
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	PLX1	PLX2	
14:0	4.4 (0.3)	8.8 (3.5)	7.6 (0.9)	18.8 (1.4)	2.9 (0.4)	3.2 (0.3)	4.9 (1.5)	7.0 (1.2)	13.1 (0.1)	9.8 (3.5)	6.2 (0.3)	6.8 (1.4)	8.4 (0.2)
16:0	9.3 (0.9)	9.0 (1.1)	5.2 (0.4)	27.7 (0.5)	10.7 (0.5)	7.1 (0.1)	16.5 (2.0)	13.2 (0.9)	7.9 (0.3)	16.0 (0.2)	18.9 (0.8)	18.7 (0.4)	11.3 (0.2)
16:1	13.1 (0.9)	10.2 (0.0)	18.2 (0.3)	31.7 (0.3)	19.3 (0.2)	18.7 (0.4)	21.5 (4.2)	19.8 (1.9)	15.5 (1.6)	20.6 (0.4)	18.7 (2.4)	18.5 (1.4)	20.4 (0.5)
16:2n-4	4.8 (0.4)	2.9 (0.8)	4.3 (0.0)	4.3 (0.6)	10.0 (0.1)	9.8 (0.0)	4.0 (0.2)	3.2 (0.0)	2.1 (0.7)	5.2 (5.1)	1.9 (1.9)	4.4 (2.1)	5.6 (0.5)
16:3n-4	13.3 (1.2)	8.4 (0.3)	7.0 (0.1)	1.1 (0.3)	28.7 (2.8)	18.8 (0.5)	1.5 (0.5)	3.0 (2.2)	1.7 (0.6)	0.0 (0.0)	6.0 (0.4)	13.5 (5.6)	10.5 (0.1)
16:4n-1	0.0 (0.0)	0.0 (0.0)	0.2 (0.0)	0.0 (0.0)	1.2 (0.1)	0.7 (0.1)	0.0 (0.0)	0.2 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.5 (0.0)
18:1n-9	0.7 (0.7)	0.6 (0.4)	0.5 (0.1)	0.9 (0.0)	0.5 (0.1)	0.9 (0.1)	3.3 (0.5)	2.4 (0.5)	2.3 (0.3)	0.0 (0.0)	6.1 (0.2)	2.5 (0.6)	0.7 (0.0)
18:1n-7	0.0 (0.0)	0.0 (0.0)	0.2 (0.0)	0.4 (0.4)	0.5 (0.2)	0.9 (0.1)	1.0 (0.1)	0.9 (0.1)	1.6 (0.0)	0.0 (0.0)	1.8 (1.8)	0.0 (0.0)	0.6 (0.0)
18:2n-6	1.2 (0.2)	0.5 (0.0)	0.5 (0.3)	0.8 (0.2)	1.3 (0.0)	1.2 (0.1)	9.2 (1.6)	7.9 (1.5)	8.1 (1.2)	8.5 (0.3)	6.0 (0.8)	3.7 (1.5)	1.6 (0.0)
20:4n-6	0.4 (0.4)	0.4 (0.0)	1.0 (0.0)	0.4 (0.1)	0.5 (0.1)	0.7 (0.0)	3.3 (0.8)	3.7 (0.6)	4.1 (0.6)	1.8 (1.8)	0.0 (0.0)	0.9 (0.9)	1.1 (0.0)
20:5n-3	15.1 (1.6)	7.1 (0.0)	44.7 (0.3)	5.5 (0.0)	17.7 (0.7)	29.9 (0.6)	16.4 (1.3)	21.6 (1.2)	31.2 (1.4)	29.8 (3.9)	20.9 (3.2)	17.3 (2.3)	27.8 (0.6)
22:6n-3	2.3 (0.3)	0.5 (0.0)	0.4 (0.0)	0.9 (0.3)	1.4 (0.0)	1.2 (0.1)	8.1 (1.5)	9.9 (1.6)	7.8 (1.4)	8.4 (0.6)	6.7 (1.4)	4.0 (1.3)	2.0 (0.1)
USCFAs ^c	33.0 (2.3)	49.5 (3.7)	8.5 (0.4)	0.6 (0.0)	0.4 (0.1)	1.2 (0.5)	0.0 (0.0)	0.4 (0.0)	0.3 (0.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.9 (0.4)
Others	2.4	2.2	2.0	6.9	4.9	5.8	10.5	6.8	4.4	0.0	6.8	9.8	3.9

^a Values are averages of three independent measurements. Standard errors are shown in parentheses.

^b MAG, DAG and TAG stated for mono-, di-, and triacylglycerols, respectively; SQD, DGD, and MGD stated for sulfoquinovosyl-, digalactosyl-, and monogalactosyldiacylglycerols, respectively; PI, PC, PG, and PE stated for phosphatidylinositol, -choline, -glycerol, and -ethanolamine, respectively; PLX are unknown phospholipids.

^c USCFAs = unknown short-chain fatty acids. For additional details see López Alonso et al. (1998).

age changed (Tables 1–4). The observed trends were a moderate increase of 16:0, 16:3, and 20:5 and a decrease in 16:1 from older (Table 1) to younger cells (Table 4). Note that 16:1 and 20:5 are the two major fatty acids in this microalga, together accounting for ca. 50% of total fatty acids. As the total fatty acid content did not change, and was always around 11% of dry weight (data not shown), the youngest cells produce a little more 16:0, 16:3, and 20:5 at the expense of 16:1. This pattern was not consistently seen in all the major acyl lipid classes. For example, for DGD, when dilution rate was increased (age decreased) 16:1 declined from 33 to 16%, 16:0 increased moderately from 10 to 14%, 20:5 decreased slightly from 13 to 8%, and 16:3 increased dramatically from 18 to 36% (Tables 1–4). Meanwhile, for the other major galactolipid, MGD, 16:1 again decreased from 28 to 18%, 16:0 increased from 6 to 12%, 20:5 content increased substantially from 9 to 25%, but 16:3 decreased from 27

to 15% (Tables 1–4). Therefore, the overall fatty acid changes elicited by cell ageing were different for different lipid classes (Tables 1–4). This also suggests that the synthesis of polyunsaturates is more active in young fast growing cells which are building up their photosynthetic apparatus made up chiefly of membranes rich in PUFAs (Gurr and Harwood, 1991). Additionally, these results suggest that DGD is the substrate for the desaturase in the synthesis of 16:3 from 16:1.

3.1.2. Lipid class composition

Average cell age had a stronger impact on lipid classes as can be seen in Fig. 1. Triacylglycerols (TAG), for instance, ranged between 42 and 69% showing the lowest value at D_3 (average cell age 1.75 days) (Fig. 1). GLs oscillated between 20 and 40% the highest value appearing at D_3 (Fig. 1). The content in polar lipids in general tended to increase at the highest

Table 4

Lipid and fatty acid composition of *P. tricornutum* cultured at dilution rate 4 ($D = 0.036 \text{ h}^{-1}$)^a

	Lipid ^b (% of fatty acids)												
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	PLX1	PLX2	Biomass
14:0	9.8 (0.0)	7.7 (2.1)	8.3 (0.0)	12.2 (1.8)	3.4 (0.3)	5.7 (0.1)	2.2 (0.1)	6.8 (1.4)	4.6 (0.9)	6.7 (1.6)	1.1 (1.1)	7.6 (0.0)	7.6 (0.2)
16:0	12.4 (0.5)	11.2 (0.7)	5.8 (0.4)	33.2 (0.2)	14.1 (0.4)	12.2 (0.5)	16.1 (0.9)	17.3 (2.0)	15.0 (0.5)	13.9 (5.4)	17.9 (5.3)	14.4 (1.9)	12.9 (0.3)
16:1	12.8 (4.1)	13.9 (0.2)	16.5 (0.1)	27.6 (2.2)	15.7 (0.9)	18.4 (0.3)	14.6 (2.5)	15.1 (3.2)	13.6 (2.3)	12.9 (4.1)	15.6 (2.6)	14.9 (0.9)	17.9 (0.9)
16:2n-4	6.0 (1.3)	4.0 (0.3)	5.0 (0.2)	10.6 (2.7)	9.0 (0.3)	7.8 (0.5)	5.2 (1.4)	3.3 (0.7)	3.1 (1.3)	7.8 (7.8)	3.6 (3.6)	5.0 (2.8)	5.5 (0.5)
16:3n-4	15.9 (1.1)	10.2 (0.0)	6.5 (0.1)	2.8 (0.1)	36.3 (0.4)	15.4 (0.3)	1.6 (0.2)	1.0 (0.0)	2.9 (0.6)	1.6 (1.5)	17.9 (17.9)	3.2 (1.2)	10.8 (0.2)
16:4n-1	1.2 (0.2)	0.7 (0.0)	0.4 (0.0)	0.0 (0.0)	3.3 (0.1)	1.4 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.5 (1.5)	0.0 (0.0)	0.9 (0.0)
18:1n-9	0.5 (0.1)	0.3 (0.3)	0.9 (0.7)	0.9 (0.0)	0.5 (0.1)	1.5 (0.8)	1.9 (0.0)	1.3 (0.1)	0.4 (0.4)	2.9 (2.9)	0.9 (0.9)	3.9 (1.8)	0.5 (0.0)
18:1n-7	0.0 (0.0)	0.0 (0.0)	0.3 (0.0)	0.7 (0.0)	0.6 (0.1)	0.6 (0.4)	0.8 (0.0)	1.0 (0.0)	0.8 (0.8)	0.9 (0.9)	0.0 (0.0)	2.6 (2.6)	0.6 (0.0)
18:2n-6	1.1 (0.1)	0.7 (0.2)	0.8 (0.1)	1.4 (0.1)	0.7 (0.1)	1.4 (0.5)	9.5 (0.1)	6.1 (0.8)	3.2 (0.2)	1.9 (1.9)	0.0 (0.0)	6.4 (4.9)	1.5 (0.0)
20:4n-6	0.8 (0.2)	0.6 (0.0)	1.0 (0.0)	0.0 (0.0)	0.2 (0.1)	0.7 (0.0)	3.8 (0.1)	3.4 (0.5)	1.8 (0.0)	1.2 (1.2)	0.0 (0.0)	0.4 (0.4)	1.1 (0.0)
20:5n-3	6.9 (0.9)	14.5 (0.3)	43.8 (0.9)	2.7 (0.2)	8.3 (1.1)	24.8 (2.4)	19.2 (0.9)	20.4 (1.8)	32.1 (13.9)	38.7 (16.5)	20.8 (13.0)	18.7 (2.8)	29.0 (0.6)
22:6n-3	2.2 (0.2)	3.4 (1.6)	0.5 (0.0)	1.7 (0.2)	1.7 (0.4)	1.5 (0.1)	14.1 (0.8)	15.9 (2.4)	19.0 (11.1)	7.2 (2.3)	13.5 (2.5)	6.3 (3.1)	2.5 (0.1)
USCFAs ^c	27.9 (7.3)	31.1 (0.1)	7.5 (0.7)	0.5 (0.5)	0.2 (0.1)	2.1 (1.0)	0.0 (0.0)	0.4 (0.0)	1.9 (1.9)	1.0 (0.9)	0.0 (0.0)	4.6 (4.6)	5.3 (0.1)
Others	2.6	1.9	2.7	5.8	6.2	6.5	11.3	8.1	1.5	3.4	7.3	12.3	4.0

^a Values are averages of three independent measurements. Standard errors are shown in parentheses.

^b MAG, DAG and TAG stated for mono-, di-, and triacylglycerols, respectively; SQD, DGD, and MGD stated for sulfoquinovosyl-, digalactosyl-, and monogalactosyldiacylglycerols, respectively; PI, PC, PG, and PE stated for phosphatidylinositol, -choline, -glycerol, and -ethanolamine, respectively; PLX are unknown phospholipids.

^c USCFAs = unknown short-chain fatty acids. For additional details see López Alonso et al. (1998).

dilution rates (youngest cells) but there was considerable fluctuations around this general trend. Extreme values for all lipids were found at D_3 which seems to represent a 'limit' steady-state. Polar lipids in general, and, particularly, main galactolipids (MGD and DGD) showed their highest values at this state. Because polar lipids are membrane lipids (Gurr and Harwood, 1991) this suggests that, at D_3 dilution rate, the cells contained a higher proportion of membranes (especially chloroplast membranes). Probably, this state represents an 'optimum' for *P. tricornutum* cells as suggested by the fact that biomass productivity reached a maximum at this dilution rate; the biomass productivity decreased when culture was moved in either direction from this optimum point (data not shown).

A reduction in polar lipids and an increase in TAG are usually associated with culture ageing (Álvarez Cobelas, 1989; Hodgson et al., 1991; Dun-

stan et al., 1993; Lombardi and Wangersky, 1995; Brown et al., 1996), however, the specific response may be different in different microalgae (Lombardi and Wangersky, 1995), or the response may depend on the steady state reached as shown in this work. When dilution rate increases (i.e. age decreases) there is an increase in polar lipids but up to a limit (D_3 in our case). If this limiting dilution rate is exceeded the trend is reversed.

These changes in lipid profiles can be attributed to two opposing factors which always change simultaneously in continuous cultures: a high dilution rate that gives rise to low biomass concentration and an increase in light availability because of a decrease in mutual shadowing of cells (Molina Grima et al., 1994). Therefore, as D is incremented, the average light a cell receives is also increased and this reduces the need for a large photosynthetic apparatus to harvest light. Increase in light availability apparently reduces the ac-

Table 5

Lipid and fatty acid composition of *P. tricornutum* cultured at dilution rate 2 ($D = 0.012 \text{ h}^{-1}$) and half nitrogen concentration (5 mmol)^a

	Lipid ^b (% of fatty acids)												Biomass
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	PLX1	PLX2	
14:0	3.9 (0.1)	6.1 (0.4)	10.5 (0.3)	7.7 (0.4)	1.4 (0.4)	5.6 (1.2)	1.7 (0.1)	2.1 (0.4)	4.4 (0.4)	0.0 (0.0)	0.0 (0.0)	4.7 (2.0)	8.1 (0.3)
16:0	19.4 (3.7)	10.8 (0.6)	7.1 (0.3)	30.3 (1.3)	9.9 (0.6)	13.1 (0.1)	22.6 (3.8)	8.0 (2.5)	7.2 (1.0)	69.5 (3.5)	62.4 (3.6)	17.6 (3.6)	12.0 (0.2)
16:1	22.5 (3.3)	14.8 (1.6)	22.7 (0.7)	31.2 (3.7)	21.1 (0.1)	19.4 (2.2)	22.8 (1.6)	14.9 (1.9)	20.7 (1.7)	14.6 (14.6)	10.5 (10.5)	13.5 (1.4)	22.5 (0.3)
16:2n-4	3.5 (1.2)	2.6 (0.3)	3.0 (0.0)	3.1 (0.2)	8.8 (0.2)	4.2 (0.6)	3.0 (1.4)	1.8 (0.0)	0.9 (0.9)	0.0 (0.0)	0.0 (0.0)	0.9 (0.8)	3.5 (0.3)
16:3n-4	11.4 (0.2)	7.3 (0.3)	6.2 (0.0)	3.7 (0.1)	38.4 (1.3)	11.7 (1.2)	8.4 (6.9)	1.6 (0.1)	8.2 (0.6)	0.0 (0.0)	0.0 (0.0)	2.3 (2.3)	9.0 (0.1)
16:4n-1	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)	0.5 (0.0)	0.0 (0.0)	0.7 (0.7)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.0)
18:1n-9	0.0 (0.0)	0.9 (0.1)	0.7 (0.1)	2.8 (0.9)	0.7 (0.1)	6.3 (3.7)	2.7 (0.0)	3.7 (0.0)	1.8 (1.8)	0.0 (0.0)	0.0 (0.0)	5.0 (0.4)	1.0 (0.0)
18:1n-7	0.0 (0.0)	0.0 (0.0)	0.4 (0.0)	0.8 (0.1)	0.5 (0.0)	0.4 (0.4)	0.6 (0.6)	1.1 (0.0)	3.7 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.6 (0.0)
18:2n-6	1.7 (0.2)	1.1 (0.3)	1.2 (0.0)	2.5 (0.5)	1.5 (0.1)	4.2 (0.9)	6.0 (0.8)	13.9 (0.8)	9.3 (0.7)	6.5 (6.5)	5.3 (5.3)	15.9 (1.9)	2.1 (0.0)
20:4n-6	0.0 (0.0)	0.0 (0.0)	0.4 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.2)	0.4 (0.4)	2.4 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.8 (0.8)	0.5 (0.0)
20:5n-3	13.0 (0.4)	11.8 (3.7)	37.6 (1.5)	3.5 (0.4)	6.8 (0.4)	17.8 (1.7)	8.7 (1.2)	21.2 (1.6)	26.1 (1.5)	9.4 (9.4)	10.1 (10.1)	15.7 (1.3)	27.9 (0.2)
22:6n-3	3.6 (0.7)	1.2 (0.2)	0.5 (0.1)	1.0 (0.4)	2.1 (0.6)	3.0 (0.6)	4.5 (0.9)	14.9 (1.7)	4.5 (4.5)	0.0 (0.0)	4.9 (4.9)	5.6 (5.6)	2.0 (0.0)
USCFAs ^c	19.0 (7.4)	41.5 (5.2)	6.6 (0.8)	0.5 (0.5)	0.2 (0.2)	1.3 (0.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	5.0 (0.1)
Others	2.1	1.8	3.0	12.9	8.2	13.1	17.9	14.3	13.2	0.0	6.8	18.1	5.6

^a Values are averages of three independent measurements. Standard errors are shown in parentheses.

^b MAG, DAG and TAG stated for mono-, di-, and triacylglycerols, respectively; SQD, DGD, and MGD stated for sulfoquinovosyl-, digalactosyl-, and monogalactosyldiacylglycerols, respectively; PI, PC, PG, and PE stated for phosphatidylinositol, -choline, -glycerol, and -ethanolamine, respectively; PLX are unknown phospholipids.

^c USCFAs = unknown short-chain fatty acids. For additional details see López Alonso et al. (1998).

cumulation of polar lipids in membranes. This occurs, for example, when D is increased from 0.024 to 0.036 h^{-1} .

3.2. Changes related with nitrogen concentration

At two dilution rates (D_2 and D_3) we assayed also the effect of nitrogen concentration on lipid composition (see Section 2). In addition to the nitrogen concentration supplied by the standard medium used, (high enough to support a nitrogen-saturated, photo-limited growth), we also investigated one-half (1/2) and one-fifth (1/5) of the standard nitrogen concentrations (Tables 5–8), as detailed earlier. At half the standard nitrogen concentration the culture was slightly deprived whereas at 1/5 of the standard concentration the culture was starved, as observed by a decrease in the steady-state biomass concentration (data not shown).

3.2.1. Fatty acid composition

At dilution rate D_2 , with the nitrogen supply reduced to 1/2 of the standard, only minor changes took place (compare Tables 2 and 5) but major ones arose when the nitrogen supply was subsequently reduced to 1/5 of the concentration in the standard medium (compare Tables 2 and 6). For example, the 16:0 content in the biomass increased from 10 to 27%, while 16:1 increased from 23 to 30%. In parallel, the 16:3 content decreased dramatically from 10 to 2%, and 20:5 decreased from 25 to 15% (Tables 2 and 6). Similar changes were observed in the main lipid classes (TAG, DGD, and MDG) where the effect was especially clear for 16:3 which diminished to a third at 1/5 nitrogen concentration with respect to the experiments carried out using the standard medium (Table 6).

At D_3 (maximum biomass productivity point) similar changes were observed when the nitrogen concen-

Table 6

Lipid and fatty acid composition of *P. tricornutum* cultured at dilution rate 2 ($D = 0.012 \text{ h}^{-1}$) and one-fifth nitrogen concentration (2 mmol)^a

	Lipid ^b (% of fatty acids)												Biomass
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	PLX1	PLX2	
14:0	5.3 (0.5)	8.5 (0.7)	9.4 (0.1)	13.7 (0.3)	3.6 (0.1)	8.5 (0.9)	5.0 (1.8)	6.2 (1.1)	11.5 (0.3)	4.8 (4.8)	7.9 (0.6)	7.9 (1.0)	8.7 (0.1)
16:0	21.7 (0.5)	19.7 (0.6)	24.3 (0.6)	36.1 (0.4)	21.2 (0.1)	16.9 (0.5)	34.8 (3.1)	13.8 (2.0)	15.6 (4.0)	25.5 (2.1)	22.2 (2.0)	14.3 (1.7)	27.3 (0.6)
16:1	20.2 (1.5)	24.2 (3.0)	30.0 (0.9)	24.3 (0.2)	23.1 (0.1)	21.8 (1.5)	20.3 (2.3)	11.0 (2.2)	14.7 (5.5)	17.4 (3.1)	20.4 (6.4)	11.3 (0.7)	30.1 (0.5)
16:2n-4	6.7 (6.7)	4.7 (3.3)	1.9 (0.9)	0.9 (0.0)	3.9 (0.1)	3.2 (0.4)	1.6 (0.6)	0.6 (0.2)	0.3 (0.3)	9.2 (9.2)	3.6 (3.6)	1.9 (0.9)	1.1 (0.0)
16:3n-4	4.8 (0.1)	3.7 (0.4)	1.9 (0.1)	1.0 (0.3)	23.0 (0.4)	6.3 (0.6)	1.2 (1.2)	0.7 (0.3)	3.2 (0.2)	13.1 (13.1)	5.6 (5.6)	0.6 (0.6)	2.3 (0.2)
16:4n-1	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)	0.7 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)
18:1n-9	2.4 (0.0)	2.6 (0.2)	3.3 (0.3)	1.8 (0.1)	1.5 (0.1)	3.3 (0.3)	4.8 (0.0)	6.7 (0.8)	6.9 (1.7)	0.0 (0.0)	2.2 (2.2)	8.7 (0.8)	3.6 (0.1)
18:1n-7	0.0 (0.0)	0.5 (0.1)	0.3 (0.1)	0.9 (0.0)	0.5 (0.2)	0.7 (0.2)	0.5 (0.5)	0.9 (0.0)	2.2 (0.6)	0.0 (0.0)	0.0 (0.0)	0.4 (0.4)	0.6 (0.0)
18:2n-6	2.1 (0.1)	1.1 (0.2)	1.0 (0.0)	1.1 (0.0)	1.1 (0.0)	3.6 (0.6)	5.7 (0.3)	10.7 (1.2)	7.4 (2.6)	0.0 (0.0)	3.5 (3.5)	7.9 (1.1)	1.5 (0.1)
20:4n-6	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (0.0)	0.0 (0.0)	1.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)
20:5n-3	11.9 (1.3)	13.8 (3.1)	18.5 (0.0)	8.1 (0.0)	8.9 (0.1)	20.7 (3.9)	11.4 (0.6)	23.6 (1.6)	22.9 (3.3)	17.7 (0.8)	19.6 (1.6)	20.9 (0.6)	14.7 (0.7)
22:6n-3	2.6 (0.3)	0.8 (0.2)	0.4 (0.0)	0.3 (0.3)	0.6 (0.2)	0.9 (0.9)	1.3 (1.3)	8.3 (0.6)	5.6 (0.3)	0.0 (0.0)	5.6 (5.6)	6.1 (1.7)	1.0 (0.1)
USCFAs ^c	5.2 (0.7)	13.4 (5.0)	2.6 (1.0)	0.2 (0.2)	0.0 (0.0)	3.6 (2.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.4 (0.4)	1.3 (0.2)
Others	17.2	7.0	6.3	11.5	11.9	10.2	13.4	16.5	9.8	12.4	9.5	19.7	7.7

^a Values are averages of three independent measurements. Standard errors are shown in parentheses.

^b MAG, DAG and TAG stated for mono-, di-, and triacylglycerols, respectively; SQD, DGD, and MGD stated for sulfoquinovosyl-, digalactosyl-, and monogalactosyldiacylglycerols, respectively; PI, PC, PG, and PE stated for phosphatidylinositol, -choline, -glycerol, and -ethanolamine, respectively; PLX are unknown phospholipids.

^c USCFAs = unknown short-chain fatty acids. For additional details see López Alonso et al. (1998).

Table 7

Lipid and fatty acid composition of *P. tricornutum* cultured at dilution rate 3 ($D = 0.024 \text{ h}^{-1}$) and half nitrogen concentration (5 mmol)^a

	Lipid ^b (% of fatty acids)												Biomass
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	PLX1	PLX2	
14:0	4.6 (0.0)	7.1 (0.4)	9.9 (0.0)	17.3 (0.0)	9.5 (0.0)	6.8 (0.0)	8.2 (0.1)	8.4 (0.5)	12.0 (1.1)	18.3 (0.0)	14.1 (0.3)	9.9 (0.1)	10.7 (0.0)
16:0	17.2 (0.1)	6.5 (0.7)	7.8 (0.1)	27.5 (0.0)	17.8 (0.1)	12.1 (0.0)	29.6 (0.3)	14.1 (0.6)	8.8 (0.8)	5.4 (0.0)	15.1 (0.1)	16.4 (0.2)	15.8 (0.1)
16:1	26.6 (0.1)	10.3 (0.1)	22.8 (0.1)	29.5 (0.0)	27.8 (0.1)	27.2 (0.2)	26.7 (1.3)	17.3 (0.5)	16.2 (0.1)	9.2 (0.3)	26.3 (0.0)	12.2 (0.4)	24.6 (0.1)
16:2n-4	4.9 (0.0)	1.3 (0.1)	2.8 (0.0)	2.3 (0.0)	3.8 (1.3)	8.5 (0.1)	0.7 (0.7)	0.9 (0.9)	7.0 (2.4)	1.2 (0.4)	0.0 (0.0)	2.8 (0.4)	3.8 (0.0)
16:3n-4	6.5 (0.0)	4.9 (0.1)	5.6 (0.1)	0.4 (0.0)	6.9 (0.0)	12.8 (0.3)	2.5 (0.0)	0.5 (0.4)	4.7 (0.4)	0.9 (0.4)	0.0 (0.0)	0.0 (0.0)	5.9 (0.1)
16:4n-1	0.9 (0.0)	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)	1.1 (0.0)	1.6 (0.0)	0.0 (0.0)	0.2 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.6 (0.0)
18:1n-9	0.0 (0.0)	0.7 (0.0)	1.3 (0.0)	0.5 (0.0)	0.6 (0.0)	1.6 (0.0)	0.0 (0.0)	1.1 (0.0)	1.3 (0.1)	1.5 (0.0)	0.0 (0.0)	6.4 (0.0)	0.7 (0.0)
18:1n-7	0.0 (0.0)	0.0 (0.0)	0.2 (0.0)	0.4 (0.0)	0.5 (0.0)	0.3 (0.0)	0.0 (0.0)	0.8 (0.0)	2.1 (0.2)	0.9 (0.0)	0.0 (0.0)	0.0 (0.0)	0.5 (0.0)
18:2n-6	0.8 (0.0)	0.3 (0.0)	0.6 (0.0)	0.7 (0.0)	1.2 (0.0)	0.8 (0.0)	2.8 (0.0)	5.2 (0.2)	4.8 (0.4)	6.5 (0.0)	3.7 (0.1)	4.1 (0.0)	1.6 (0.0)
20:4n-6	0.0 (0.0)	0.0 (0.0)	0.2 (0.0)	0.0 (0.0)	0.2 (0.0)	0.1 (0.0)	0.0 (0.0)	0.9 (0.0)	0.0 (0.0)	1.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (0.0)
20:5n-3	12.0 (0.4)	2.7 (0.0)	42.6 (0.3)	6.0 (0.0)	20.9 (0.7)	17.7 (0.1)	12.8 (0.0)	26.6 (1.4)	19.9 (2.1)	37.9 (0.4)	29.6 (0.5)	19.5 (1.0)	22.5 (0.1)
22:6n-3	13.4 (0.1)	2.4 (0.0)	0.6 (0.0)	6.9 (0.0)	1.5 (0.0)	0.7 (0.0)	5.1 (0.0)	12.7 (4.2)	12.9 (7.5)	7.0 (0.1)	3.6 (0.3)	5.9 (0.1)	2.2 (0.0)
USCFAs ^c	4.4 (0.0)	60.9 (0.4)	2.3 (0.4)	0.4 (0.0)	0.7 (0.1)	1.9 (0.2)	1.0 (1.0)	0.5 (0.2)	1.2 (0.1)	0.8 (0.2)	0.0 (0.0)	8.4 (1.0)	3.9 (0.0)
Others	8.8	3.1	3.3	8.2	7.7	8.0	10.6	10.8	9.2	9.5	7.6	14.4	7.0

^a Values are averages of three independent measurements. Standard errors are shown in parentheses.^b MAG, DAG and TAG stated for mono-, di-, and triacylglycerols, respectively; SQD, DGD, and MGD stated for sulfoquinovosyl-, digalactosyl-, and monogalactosyldiacylglycerols, respectively; PI, PC, PG, and PE stated for phosphatidylinositol, -choline, -glycerol, and -ethanolamine, respectively; PLX are unknown phospholipids.^c USCFAs = unknown short-chain fatty acids. For additional details see López Alonso et al. (1998).

tration was decreased. The fatty acids found in the biomass showed a notable increase in 16:0 and 16:1 with decrease of 16:2, 16:3 and 20:5 when nitrogen concentration decreased (compare Tables 3 and 8). For example, 16:0 increased from 11 to 20% of total fatty acids. These changes were even more extensively reflected in some GLs. For example, the decrease was especially large for 16:3, a fatty acid mainly located in GLs (López Alonso et al., 1998): 16:3 was reduced from 29 to 3% in DGD as the nitrogen concentration was changed from normal to one-fifth the normal value (Tables 3 and 8).

Similar responses to N-deprivation have been reported by others (Pohl and Zurheide, 1979; Piorreck and Pohl, 1984, Piorreck et al., 1984). Similar behaviour has been observed also when nutrients other than N become limiting (Reitan et al., 1994) although no detailed analysis of lipid classes has been reported in this case. Apparently nitrogen deprivation triggers a response that leads to a reduction of biomass gener-

ation while the metabolism is redirected to the synthesis of reserve components, namely saturated fatty acids, thus preparing the cells for a starvation period.

3.2.2. Lipid class composition

The most important changes caused by the decrease in nitrogen supply were observed at dilution rate D_2 and were mainly in the GL classes (Fig. 2). GL fraction decreased from 21 to 12% and both of the main GL classes, MGD and DGD, exhibited a similar trend. DGD, for example, decreased from 12 to 5% (Fig. 2). Conversely, NLs and PLs increased in parallel from about 73 to 79% and from 6 to 8%, respectively (Fig. 2). TAG was the lipid class with the highest increase, from 69 to 75% (Fig. 2).

It is well documented that most microalgae increase their total lipid content in response to N-deprivation (Shifrin and Chisholm, 1980; Piorreck and Pohl, 1984; Álvarez Cobelas, 1989; Ahlgren et al., 1992). Although there are only a few studies on detailed changes in

Table 8

Lipid and fatty acid composition of *P. tricornutum* cultured at dilution rate 3 ($D = 0.024 \text{ h}^{-1}$) and one-fifth nitrogen concentration (2 mmol)^a

	Lipid ^b (% of fatty acids)												Biomass
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	PLX1	PLX2	
14:0	22.2 (6.7)	13.7 (0.3)	10.2 (0.3)	15.2 (0.0)	11.8 (0.0)	4.9 (0.0)	5.8 (0.1)	6.8 (0.6)	9.4 (2.5)	20.1 (0.1)	11.8 (0.3)	7.1 (0.0)	11.3 (0.0)
16:0	13.4 (2.0)	14.3 (1.0)	9.1 (0.1)	34.7 (0.0)	19.1 (0.0)	10.9 (0.0)	30.9 (1.4)	15.5 (1.7)	9.3 (2.3)	8.5 (0.0)	21.2 (0.5)	16.1 (0.1)	19.5 (0.0)
16:1	22.6 (3.3)	24.3 (1.1)	20.6 (0.4)	35.4 (0.0)	26.2 (0.1)	19.3 (0.0)	26.0 (0.5)	13.6 (1.5)	14.9 (2.6)	8.5 (0.0)	33.7 (0.7)	7.4 (1.7)	28.1 (0.3)
16:2n-4	2.0 (0.2)	1.9 (0.1)	1.8 (0.1)	3.2 (0.0)	6.1 (2.0)	6.8 (0.1)	1.9 (1.2)	1.2 (0.2)	2.4 (2.4)	1.1 (0.0)	1.2 (0.1)	1.9 (0.7)	2.9 (0.3)
16:3n-4	3.0 (0.9)	3.7 (0.3)	2.9 (0.6)	0.0 (0.0)	2.8 (1.9)	11.3 (0.0)	0.0 (0.0)	0.4 (0.1)	0.0 (0.0)	0.0 (0.0)	0.7 (0.0)	0.6 (0.6)	2.9 (0.0)
16:4n-1	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)	1.1 (0.0)	2.3 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.5 (0.0)
18:1n-9	1.6 (0.2)	1.1 (0.0)	1.1 (0.0)	0.5 (0.0)	0.6 (0.0)	2.2 (0.0)	0.7 (0.0)	0.9 (0.1)	0.0 (0.0)	1.0 (0.0)	2.9 (0.2)	6.9 (0.1)	1.3 (0.0)
18:1n-7	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)	0.4 (0.0)	0.3 (0.0)	0.0 (0.0)	0.4 (0.0)	0.0 (0.0)	0.6 (0.0)	0.0 (0.0)	0.0 (0.0)	0.4 (0.0)
18:2n-6	0.9 (0.1)	0.5 (0.0)	0.6 (0.0)	0.8 (0.0)	1.2 (0.0)	0.8 (0.0)	3.7 (0.3)	4.5 (0.5)	2.6 (0.7)	5.4 (0.0)	2.2 (0.3)	1.6 (0.0)	1.5 (0.0)
20:4n-6	0.0 (0.0)	0.0 (0.0)	0.3 (0.0)	0.0 (0.0)	0.4 (0.0)	0.0 (0.0)	0.0 (0.0)	0.4 (0.0)	0.0 (0.0)	0.6 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)
20:5n-3	7.5 (0.4)	3.3 (0.1)	48.0 (2.0)	4.3 (0.3)	19.6 (0.0)	22.0 (0.1)	13.2 (1.0)	27.2 (3.0)	34.8 (8.8)	38.0 (0.2)	13.0 (1.0)	8.0 (0.1)	20.0 (0.1)
22:6n-3	6.9 (4.9)	1.7 (1.1)	0.2 (0.0)	0.5 (0.0)	2.0 (0.0)	0.8 (0.0)	5.0 (0.2)	15.3 (6.1)	19.7 (3.4)	6.2 (0.0)	2.2 (0.0)	1.6 (1.0)	1.9 (0.0)
USCFAs ^c	5.7 (1.5)	29.2 (2.5)	2.2 (0.1)	0.0 (0.0)	0.6 (0.0)	2.5 (0.1)	0.2 (0.2)	0.3 (0.0)	0.0 (0.0)	0.5 (0.5)	2.0 (0.0)	5.5 (0.1)	2.2 (0.0)
Others	14.2	6.2	3.1	5.6	8.2	16.2	12.6	13.6	6.8	9.6	9.2	43.4	7.6

^a Values are averages of three independent measurements. Standard errors are shown in parentheses.^b MAG, DAG and TAG stated for mono-, di-, and triacylglycerols, respectively; SQD, DGD, and MGD stated for sulfoquinovosyl-, digalactosyl-, and monogalactosyldiacylglycerols, respectively; PI, PC, PG, and PE stated for phosphatidylinositol, -choline, -glycerol, and -ethanolamine, respectively; PLX are unknown phospholipids.^c USCFAs = unknown short-chain fatty acids. For additional details see López Alonso et al. (1998).

lipid classes, the depletion of polar lipids and the increase of NL seem to be a general response to N-stress in microalgae (Pohl and Zurheide, 1979; Regnault et al., 1995). Similar trends have been reported for *P. tricornutum* in batch cultures (Parrish and Wangersky, 1987) although deficiencies in the analytical procedures followed in that work precluded a deeper discussion. At dilution rate D_3 the picture was dramatically changed and a decrease in the nitrogen concentration in the fresh medium caused the NLs to diminish from 50 to 28% while GLs increased from 39 to 51% (Fig. 3). The other polar lipid fraction, PLs, was also significantly increased from 11 to 21%. Therefore, surprisingly, the polar lipids increased from 50 to 72% when nitrogen concentration decreased from the standard value to 1/5 (Fig. 3). This effect was especially marked in DGD which increased from 15 to 36% while the other GLs, MGD and SQD, slightly decreased when N-content was reduced (Fig. 3). This

behaviour at dilution rate D_3 conflicts with the usual one reported in the references cited above and with our own results for D_2 .

If observations are made at the level of dry weight content we can consider the exact nature of changes. At D_2 , acyl lipids increased from 11 to 19% of biomass dry weight when nitrogen concentration decreased. Conversely, at D_3 , the acyl lipid content was nearly undisturbed (ca. 11% of dry weight) by the changes in nitrogen concentration (data not shown). Therefore, at D_2 , the GL changes described in percentage of total lipids were mostly 'apparent', in fact GLs slightly increased from 2.0 to 2.2% of dry weight, when nitrogen concentration decreased (data not shown). However, at the same time, NLs increased from 8.4 to 14.6% of dry weight (data not shown) representing a *relative* decrease of GLs. Conversely, at D_3 , both changes, i.e. decrease in NLs and increase in GLs were 'real' in terms of dry weight. A biological ex-

planation for this phenomenon is lacking. Apparently young cells seem to respond to N-limitation by improving the system (membranes) of N-fixation.

These observations illustrate that average cell age (dilution rate) and nitrogen availability interact. Hence, the common assertion that N-starvation increases lipid content in microalgae (Pohl and Zurheide, 1979; Shifrin and Chisholm, 1980; Piorreck and Pohl, 1984; Piorreck et al., 1984; Álvarez Cobelas, 1989) is not always true; the effect of N-availability depends also on cell age as shown here. N-starvation may increase or may leave unchanged the total lipid content depending on the dilution rate. This supports the suggestion that ageing effects and nutrient limitation effects cannot be discriminated, or may be confused (Álvarez Cobelas, 1989) in batch cultures. Moreover, the effect may be quite different on the different lipid classes depending on dilution rate. What seems to be well supported is that N-starvation always decreases the amount of polyunsaturated fatty acids.

Summarising, fatty acid composition of *P. tricornutum* biomass was relatively more stable than lipid class composition; in other words, *P. tricornutum* reacts to changes in culture conditions mostly by retailoring its lipid classes and to a lesser extent, altering its fatty acid composition. The direction of the changes in lipid composition, elicited by environmental changes, cannot be clearly predicted because culture factors interact in an unpredictable way. Also, as we have shown, the response of a microalgal culture to a factor (cell age or nitrogen concentration) is affected by the type of culture (batch or continuous).

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