N/P ratio effect on lipid profiles of native marine microalgae and their potential for sustainable bioproducts

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Abstract: The increasing demand for energy and growing environmental concerns have accelerated the search for alternative energy sources, with microalgae emerging as a promising candidate. These photosynthetic organisms can produce high-value biomolecules suitable for biofuel production and other industrial applications. However, their utility largely depends on biomass composition, which is influenced by nutrient availability—particularly phosphorus (P) and nitrogen (N). This study investigates the impact of varying N/P ratios (9, 12, and 14) on the lipid content and lipid profiles of marine microalgae strains *Nannochloropsis* (NSRE-1, NSRE-2) and *Nannochloris* (NRRE-2), native to the Yucatán coast of Mexico. The potential use of the extracted lipids in biofuel and value-added product development is also evaluated. Results indicate a significant decline in lipid content across all three strains with increasing N/P ratios ($p < 0.05$). Notably, strain NSRE-1 achieved the highest relative fatty acid content (69.9%) at an N/P ratio of 14, while fatty acid synthesis in NRRE-2 was inhibited at N/P ratios of 12 and 14. These findings underscore the critical role of the N/P ratio in modulating lipid production and composition in microalgae, particularly in endemic strains. Moreover, changes in the N/P ratio stimulated the production of lipophilic compounds with potential applications in bioplasticizer manufacturing. Overall, the useof biofuels and naturally derived plasticizers supports the rising industrial demand for sustainable, renewable alternatives to fossil fuels and conventional plastics.

Keywords: marine microalgae, biofuels, bioplasticizers, N/P ratio, lipid metabolism

Introduction

Over the past several decades, increasing energy demands and escalating environmental challenges have intensified the search for alternative renewable energy sources, including those derived from microalgae (Duarah *et al*., 2022). Microalgae synthesize a wide variety of biomolecules with applications across the pharmaceutical, cosmetic, energy, and aquaculture industries (Borowitzka, 2013). Among microalgae-based biofuels, biodiesel is particularly promising due to its potential to significantly reduce greenhouse gas emissions, including CO_2 and sulfur oxides (SO_x). Microalgal biomass presents several advantages as a biofuel feedstock, such as high photosynthetic efficiency, rapid growth, and high tolerance to environmental contaminants (Li *et al*., 2022). Notably, microalgae can produce lipid yields that exceed those of terrestrial plants by 15- to 300-fold on a dry weight basis (Chisti, 2008).

Microalgal lipid productivity is influenced by numerous physicochemical factors, including light intensity, photoperiod, temperature, and salinity. Nutrient availability—specifically nitrogen (N) and phosphorus (P)—plays a central role in shaping cellular biochemical composition and lipid accumulation (Sharma *et al*., 2012). The nitrogen-to-phosphorus (N/P) ratio is therefore critical for regulating cellular composition, metabolic activity, growth dynamics, and photosynthetic efficiency (Slinksienė *et al*., 2022). For instance, an N/P ratio of 18 maximized chlorophyll content in *Chlorella*, enhancing photosynthetic performance and biomass yields (Beuckels *et al*., 2015). Numerous studies have demonstrated that nutrient stoichiometry directly affects both total lipid content and the distribution of lipid classes.
Triacylglycerols (TAGs) are the most relevant lipid fraction for biodiesel production due to their lower degree of unsaturation and higher conversion efficiency compared with saturated fatty acids (D'Alessandro & Antoniosi, 2016). These characteristics translate into favorable physicochemical properties, such as appropriate viscosity, reduced pour point, and overall improved biodiesel quality. Species such as *Chlorella sp.*, *Scenedesmus sp.*, and *Nannochloropsis sp.* have been widely studied for this purpose because of their high lipid content (32–38%) and advantageous fatty acid composition (Chowdhury *et al*., 2019). While many studies have evaluated the effect of environmental factors on microalgal lipid production, limited attention has been given to the potential value-added applications of lipid fractions unsuitable for biodiesel.

This study examines the influence of three N/P ratios (9, 12, and 14) on the lipid content and lipid profile of three native marine microalgae isolated from the Yucatán coast. Furthermore, the potential use of these lipids for biofuel production and other value-added bioproducts is assessed, broadening the scope of their biotechnological applications.

Materials and Methods

Biomass Cultivation

Microalgae strains used in this study were isolated from the coastal area of Puerto Progreso, Yucatán, and identified by López-Rosales *et al*. (2020) as *Nannochloropsis* (NSRE-1 and NSRE-2) and *Nannochloris* (NRRE-1). Cultures were grown in a medium composed of 50% distilled water and 50% seawater, supplemented with 51.02 ± 0.78 mg/L of N-NO_3^-, with an initial pH of 7.06 and a salinity of 22.46 ± 0.85‰ (~20 g/L NaCl).

The N/P ratio was adjusted by adding 100 mg/L of P-PO_4^{3-} in the form of KH_2PO_4 (Sigma Aldrich, St. Louis, MO, USA). Cultures were maintained under continuous illumination at 90 µmol/m^2/s using a 30-W white light source, at 25 ± 2 °C for 22 days. Biomass harvesting was performed following the flocculation method described by Rojo-Cebreros *et al*. (2016), adjusting the pH with 0.5 N NaOH. Samples were centrifuged at 4728 g for 10 min (HERMLE Z 206 A), the supernatant was discarded, and the biomass was washed twice with distilled water to remove residual salts. The resulting pellet was frozen at −20 °C and subsequently lyophilized (Freezone, LABCONCO).

Lipid Extraction

Total lipids were extracted in duplicate using the Folch method (Folch *et al*., 1957). Approximately 38 mg of lyophilized biomass was mixed with chloroform:methanol (2:1, v/v) and processed at 40 °C and 150 rpm for 3 h. The extract was then separated from the residual biomass using a separation funnel. The organic phase was collected and evaporated to obtain the total lipid extract, which was redissolved in chloroform.

Transesterification

Fatty acid methyl esters (FAME) were obtained following the procedure of López-Rosales *et al*. (2019). Total lipids were fractionated by column chromatography using silica gel (Kieselgel, 70–230 mesh). Elution was performed using a solvent gradient consisting of hexane; hexane:ethyl acetate (9:1, 8:2, 7:3); and methanol.

Triacylglycerol-rich fractions (hexane:ethyl acetate, 9:1) were subjected to transesterification using 6% (w/v) sodium methoxide at 60 °C for 90 min in a 25-mL vial. The reaction mixture was passed through silica gel to remove excess catalyst and glycerol.

Data Analysis

All graphs and statistical analyses were carried out using Origin Pro 2018 (OriginLab Corporation). Results are reported as mean ± standard deviation. Total lipid content and relative fatty acid composition were evaluated using two-way ANOVA, considering the N/P ratio and the metabolic response of microalgae (e.g., growth and biomass accumulation) as factors. Statistical significance was defined as $P < 0.05$.

Results and Discussion

Effect of the N/P Ratio on Lipid Content

Figure 1 presents the lipid content (%) of NSRE-1, NSRE-2, and NRRE-1 under N/P ratios of 9, 12, and 14. An increase in the N/P ratio resulted in a consistent decline in lipid accumulation in all strains. Maximum lipid contents were observed at an N/P ratio of 9, reaching 6.21 ± 0.44% for NSRE-1, 7.56 ± 0.28% for NSRE-2, and 8.09 ± 0.10% for NRRE-1. Among the three strains, NRRE-1 exhibited the highest lipid content across all N/P ratios evaluated.

A two-way ANOVA confirmed that the N/P ratio had a significant effect on lipid content in NSRE-1, NSRE-2, and NRRE-1 ($P < 0.05$).

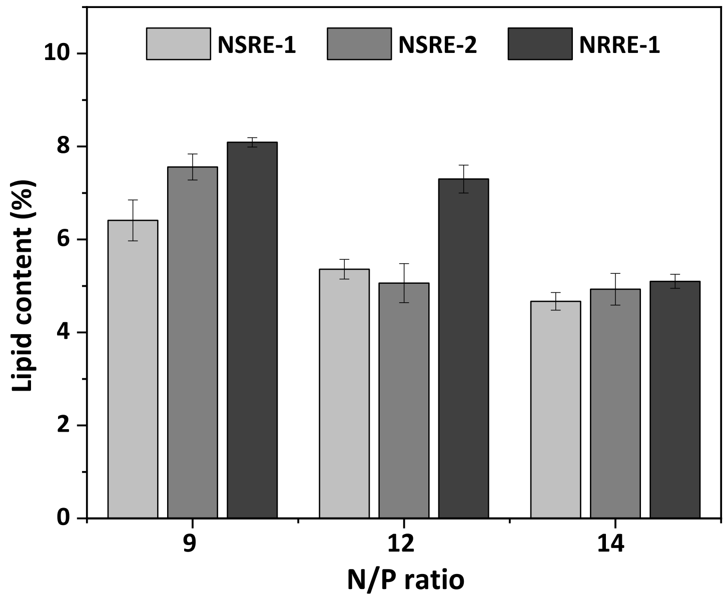


Figure 1. Total lipid content (%, mean ± SD) of the microalgal strains NSRE-1, NSRE-2, and NRRE-1 cultivated under N/P ratios of 9, 12, and 14.

Ding *et al*. (2024) reported comparable trends in *Chlorella* sp. and *Phaeodactylum tricornutum* cultivated in aquaculture wastewater. In their study, lipid content decreased at N/P ratios of 3, 6, and 12, whereas an increase to an N/P ratio of 18 resulted in substantial lipid accumulation—112.34% and 136.92%, respectively. However, lipid content declined again when the N/P ratio increased further to 30 and 36.

Previous studies indicate that under nutrient-rich conditions, microalgal cells predominantly synthesize proteins and nucleic acids, while under nitrogen-sufficient conditions (0.0–0.5 g/L), they tend to accumulate carbohydrates rather than lipids (Tossavainen *et al*., 2019). Accordingly, the reduction in lipid content observed in NSRE-1, NSRE-2, and NRRE-1 at N/P ratios above 9 suggests that higher N/P availability may have favored carbohydrate biosynthesis over lipid accumulation.

Effect of N/P Ratio on the Lipid Profile

Figure 2 presents the relative abundance of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in NSRE-1, NSRE-2, and NRRE-1 cultured under N/P ratios of 9, 12, and 14. In NSRE-1, SFA levels increased with increasing N/P ratio, reaching a maximum of 69.9% at N/P = 14. Conversely, MUFA and PUFA fractions decreased as the N/P ratio increased (Figure 2a).

The biomass of NSRE-2 showed a similar trend, as the content of SFA increased with rising N/P ratio. However, in this strain, an increase in N/P ratio also resulted in higher proportions of MUFA and PUFA. Likewise, increasing the N/P ratio reduced the content of organic compounds other than fatty acids. The maximum relative content of SFA in NSRE-2 was 20.2%, a value considerably lower than that observed in NSRE-1 (Figure 2b).

In contrast, NRRE-1 exhibited high sensitivity to changes in the N/P ratio of the culture medium (Figure 2c). Under N/P ratios of 12 and 14, this strain did not promote fatty acid synthesis; instead, it primarily produced other lipophilic compounds, particularly phthalates and adipates.

Rasdi *et al*. (2014) reported similar behavior in *Tisochrysis lutea* and *Nannochloropsis oculata* grown in F/2 medium at six N/P ratios (5, 10, 20, 30, 60, and 120). Their findings indicated that an N/P ratio of 20 enhanced microalgal growth and protein content, whereas an N/P ratio of 120 reduced both parameters but increased lipid accumulation in both strains. For *N. oculata*, an N/P ratio of 20 favored the production of eicosapentaenoic acid (EPA), while in *T. lutea*, an N/P ratio of 30 promoted the synthesis of docosahexaenoic acid (DHA). Both fatty acids are ideal raw materials for biofuel production. Similarly, Gao *et al*. (2023) reported that an N/P ratio of 18 enhanced optimal growth of *Scenedesmus obliquus* in wastewater, as well as its dry biomass, lipid production, and chlorophyll accumulation (1.70 g/L, 0.49 g/L, and 7.43 mg/L, respectively).

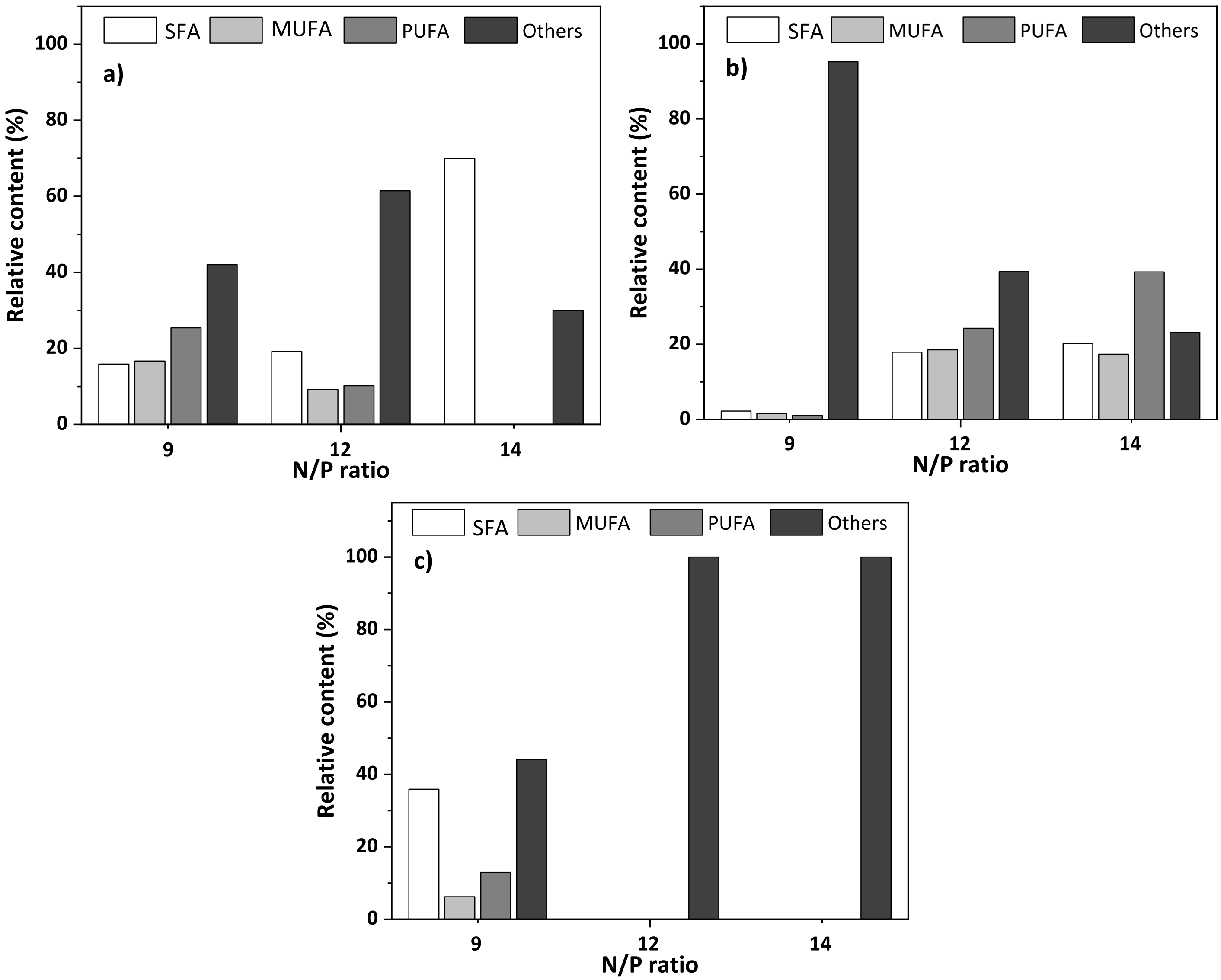


Figure 2. Relative abundance of saturated (SAF), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in the biomass of (a) NSRE-1, (b) NSRE-2, and (c) NRRE-1 cultivated under N/P ratios of 9, 12, and 14

A two-way ANOVA revealed that both the strains and the N/P ratio had significant effects on the relative content of SFA, MUFA, PUFA, and non-fatty-acid lipophilic compounds ($p < 0.05$), mainly phthalates and adipates.

Tables 1, 2, and 3 list the fatty acids identified in the biomass of NSRE-1, NSRE-2, and NRRE-1 grown under N/P ratios of 9, 12, and 14. The fatty acids produced by all strains represent an adequate feedstock for biodiesel production, as they include C16–C18 fatty acids (Chowdhury *et al*., 2019). The relatively low concentrations of saturated and long-chain polyunsaturated fatty acids such as C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), and C18:3 (linolenic acid) confer high oxidative stability and favorable thermodynamic properties.

Table 1. Fatty acid profile of NSRE-1 at N/P ratios of 9, 12, and 14.

Fatty acid	Carbon number:saturations	Relative content (%)
N/P 9		
Hexadecanoic Acid	C17:0	13.5
Methyl stearate	C19:0	2.46
Octadecenoic acid	C19:1	16.6
Octadecadienoic acid	C19:2	17.46
Linoleic acid	C20:2	7.98
N/P 12		
Hexadecanoic Acid	C17:0	15.4
Methyl stearate	C19:0	3.71
Octadecenoic acid	C19:1	9.2
Octadecadienoic acid	C19:2	10.17
N/P 14		
Hexadecanoic Acid	C17:0	53.8
Methyl stearate	C19:0	11.3
Methyl butyl hexadecanoate	C21:0	4.9

Table 2. Fatty acid profile of NSRE-2 at N/P ratios of 9, 12, and 14.

Fatty acid	Carbon number:saturations	Relative content (%)
N/P 9		
Hexadecanoic Acid	C17:0	1.7
12-Methyl tetradecanoic acid	C16:0	0.45
Octadecenoic acid	C19:1	1.55
Octadecadienoic acid	C19:2	1.04
N/P 12		
Hexadecanoic Acid	C17:0	13.3
Methyl stearate	C19:0	3.4
2-Methylundecanoic acid	C13:0	1.05
Octadecenoic acid	C19:1	18.5
Octadecadienoic acid	C19:2	16.68
Linoleic acid	C20:2	5.08
Eicosatrienoic acid	C21:3	2.48
N/P 14		
Hexadecanoic Acid	C17:0	15.6
Methyl stearate	C19:0	3.47
Dodecanoic acid	C14:0	1.08
Octadecenoic acid	C19:1	17.5
Octadecadienoic acid	C19:2	23.44
Linoleic acid	C20:2	10.05
Octadecatrienoic acid	C19:3	5.77

Table 3. Fatty acid profile of NRRE-1 at N/P ratios of 9, 12, and 14.

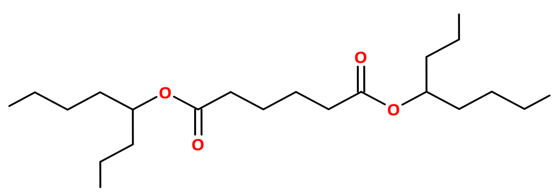
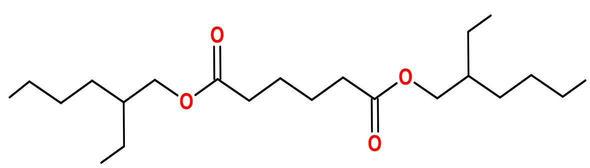
Fatty acid	Carbon number:saturations	Relative content (%)
N/P 9		
Hexadecanoic Acid	C17:0	30.8
Tetradecanoic acid	C17:0	5.2
Dodecanoic acid	C13:1	6.22
Octadecadienoic acid	C19:2	12.9

Effect of the N/P Ratio on the Synthesis of Value-Added Compounds

The lipid extracts of NSRE-1, NSRE-2, and NRRE-1 were found to contain organic compounds suitable for the production of various value-added products, including adipates and phthalates. Adipates are widely used in the cosmetic industry as plasticizers and solvents in formulations such as nail polishes, tanning lotions, liquids, and gels, typically at low concentrations (5–8%) (Chi *et al*., 2019). They are also found in food packaging materials and films at concentrations of a few thousand micrograms per gram (Vimalkumar *et al*., 2022). Unlike other plasticizers, adipates exhibit very low toxicity and are readily biodegradable (EPA, 2021).

Because adipates can be synthesized biologically, they represent promising alternatives to synthetic plasticizers. In this study, NSRE-1, NSRE-2, and NRRE-1 were observed to produce two adipates—under specific N/P ratios—that are commonly used in the manufacture of plastics (see Table 4).

Table 4. Adipates synthesized by NSRE-1, NSRE-2, and NRRE-1 at different N/P ratios and their relative content

Name	Structure	Strain	N/P ratio	Relative content (%)
Di(4-octyl) adipate		NSRE-1	14	18.5
		NSRE-2	14	10
Bis(2-ethylhexyl) hexanedioate (Di(2-ethylhexyl) adipate)		NSRE-2	12	27.8
		NRRE-1	12	34.3
		NRRE-1	14	19

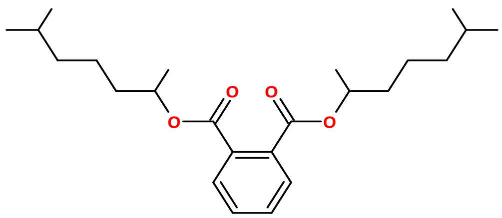
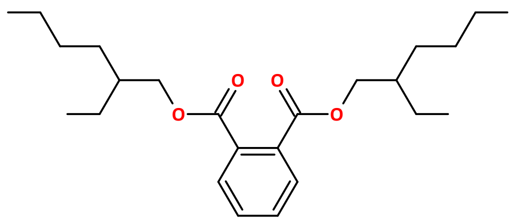
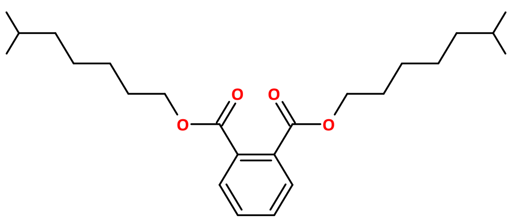
Phthalic acid esters (PAEs), or phthalates (Table 5), were also identified. Phthalates are a class of lipophilic chemical substances widely used as plasticizers and additives to improve the mechanical extensibility and flexibility of various food products. Currently, synthetic PAEs are considered potentially hazardous to ecosystems and to public health. However, PAEs are also naturally produced by microorganisms such as algae, bacteria, fungi, and by higher plants through organic extracts, root exudates, and essential oils.

PAEs are synthesized by several algal species, where they play a role in adaptation to biotic and abiotic stress (Tian *et al*., 2016). Naturally occurring PAEs include di-n-butyl phthalate, diethyl phthalate, dimethyl phthalate, di(2-ethylhexyl) phthalate, diisobutyl phthalate, and diisooctyl phthalate. Natural PAEs may function as allelochemicals, antibiotics, or insecticides.

Importantly, the natural synthesis of PAEs implies that certain microorganisms are capable of degrading them, which suggests a potential contribution to reducing environmental contamination caused by synthetic PAEs. Numerous studies have investigated microorganisms capable of degrading PAEs, and results demonstrate that several species of bacteria and microalgae possess PAE-degrading capabilities (Hu *et al*., 2021).

PAEs produced by microalgal species hold significant potential for sustainable applications. For instance, they can be incorporated into bioplastic formulations (e.g., PLA, PHB, alginates) to produce materials with improved flexibility and mechanical strength. These bioplastics may have valuable applications in the pharmaceutical, cosmetic, food, and agricultural industries. Moreover, they are considered sustainable compounds, as they can be produced from renewable biomass and tailored according to the microalgal species and cultivation conditions used.

Table 5. Relative content of phthalates synthesized by NSRE-1, NSRE-2, and NRRE-1 under different N/P ratios

Name	Structure	Strain	N/P ratio	Relative content (%)
Bis(6-methylhept-2-yl) phthalate		NSRE-1	9	17.4
Bis(2-ethylhexyl) phthalate		NSRE-1	12	47.7
		NRRE-1	14	80.1
Diisooctyl phthalate		NSRE-2	9	33.9

Conclusions

The results indicate that the N/P ratio influences both the lipid content and the lipid profile of the microalgae. Therefore, it is essential to assess how culture conditions affect the biochemical profile of microalgal strains, particularly when they are endemic.

Furthermore, specific N/P ratios were identified that promote the synthesis of hydrocarbon chains with suitable length and saturation for the production of fatty acid methyl esters. The detection of plasticizer compounds at certain N/P ratios also suggests that their presence can be selectively enhanced under specific cultivation conditions.

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Author contributions: R.M.G.B: writing, analysis, and interpretation of data; J.C.S.R: analysis and editing; T.T.T: provide materials; R.V.O.: editing, supervision, and funding acquisition.

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