

## Letter

## Evidence of nitro-fatty acids in microscopic algae and cyanobacteria

Barbora Papoušková<sup>a</sup>, Daniel Chrenko<sup>b</sup>, Pavel Hrouzek<sup>c</sup>, Martina Zatloukalová<sup>d</sup>,  
Jiří Pospíšil<sup>b,e,f</sup>, Jan Vacek<sup>d,\*</sup>

<sup>a</sup> Department of Analytical Chemistry, Faculty of Science, Palacký University, 17. listopadu 12, Olomouc, 771 46, Czech Republic

<sup>b</sup> Department of Organic Chemistry, Faculty of Science, Palacký University, 17. listopadu 1192/12, Olomouc, 771 46, Czech Republic

<sup>c</sup> Centre Algatech, Institute of Microbiology, Academy of Science of the Czech Republic, Trebon, Czech Republic

<sup>d</sup> Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University, Hnevotinska 3, Olomouc, 775 15, Czech Republic

<sup>e</sup> Department of Chemical Biology, Faculty of Science, Palacký University, Slechtitelu 27, Olomouc, 783 71, Czech Republic

<sup>f</sup> The Laboratory of Growth Regulators, Czech Academy of Sciences, Institute of Experimental Botany & Palacký University, Slechtitelu 27, Olomouc, 783 71, Czech Republic

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## ABSTRACT

Nitro-fatty acids (NO<sub>2</sub>FAs) are pleiotropic cell signalling modulators, playing a role in stress responses, development, and defence mechanisms in many organisms, including plants. However, to date their production in photosynthetic microorganism has not been studied. In this study, using LC-MS analysis, we first evaluated the ability of the green algae *Desmodesmus communis*, *D. subspicatus* and *Chlorella* sp., cyanobacterium *Synechocystis* sp., and eustigmatophyte *Trachydiscus minutus* to produce NO<sub>2</sub>FAs. It was found that the studied strains are able to produce precursor oleic acid (OA), linoleic acid (LA) and its conjugated version (cLA), as well as arachidonic acid (AA) and also stearic acid (SA). Simultaneously, we demonstrated the production of NO<sub>2</sub>OA in *Synechocystis* sp. and *D. subspicatus*, and also NO<sub>2</sub>SA in *Chlorella* sp. and *T. minutus* based on LC-MS analysis with NO<sub>2</sub>FA ultrapure regio/stereoisomeric standards. The levels of NO<sub>2</sub>OA and NO<sub>2</sub>SA were more than 1000 times lower than the level of non-nitrated FAs. These results show that freshwater algae and cyanobacteria are capable of producing saturated and unsaturated NO<sub>2</sub>FAs. The detailed distribution of NO<sub>2</sub>FAs and their function in other algae and cyanobacteria species should be clarified in the future.

## 1. Introduction

Nitro-fatty acids (NO<sub>2</sub>FAs) are pleiotropically acting compounds that occur endogenously under physiological and pathophysiological conditions in mammals. NO<sub>2</sub>FAs and related lipids are formed due to increased levels of reactive nitrogen and oxygen species during cardiovascular (Mollenhauer et al., 2018), pulmonary (Cui et al., 2006; Reddy et al., 2012), and neurodegenerative diseases (Di Maio et al., 2023) and generally inflammatory conditions (Cui et al., 2006). The presence of the nitro group gives unsaturated FAs electrophilic properties, and they can react with nucleophilic targets, especially Cys, His and other basic amino acid residues of proteins, via Michael addition (Baker et al., 2007; Grippo et al., 2021). Based on the specific electrophile-nucleophile interaction, NO<sub>2</sub>FAs have the ability to bind to key proteins (nitro-alkylation) and thereby modulate cellular responses. In general, NO<sub>2</sub>FAs inhibit nuclear factor  $\kappa$ -B (NF- $\kappa$ B), activate the Nrf2 antioxidant signalling pathway and modulate many other cellular targets, including

PPAR $\gamma$  (peroxisome proliferator-activated receptor) (Khoo et al., 2018; Roos et al., 2024).

Several NO<sub>2</sub>FAs can also act as hydrophobically stabilized nitric oxide (NO) donors in animals and plants (Aranda-Caño et al., 2026; Zatloukalova et al., 2025). Nitro-linolenic acid has been reported to be able to release NO (Mata-Pérez et al., 2016a, 2016b), which interacts with glutathione to form S-nitrosoglutathione *in vitro* and *in vivo* in *Arabidopsis* (Mata-Pérez et al., 2020). Although the presence and biological functions of NO<sub>2</sub>FAs have been extensively studied in mammalian models, little is known about the function of these molecules in plants. In plants, mainly nitro-linoleic (NO<sub>2</sub>LA) and nitro-oleic (NO<sub>2</sub>OA) acids have been detected endogenously. One of the first reports of the occurrence of NO<sub>2</sub>FAs was a study on conjugated nitro-linoleic acid (NO<sub>2</sub>cLA) in extra virgin olive oil (Fazzari et al., 2014). Later, the occurrence of nitro-linolenic acid and its effect on defence mechanisms in *Arabidopsis thaliana* was studied (Aranda-Caño et al., 2019; Mata-Pérez et al., 2017). Subsequently, nitro-linolenic acid was detected in

\* Corresponding author.

E-mail address: [jan.vacek@upol.cz](mailto:jan.vacek@upol.cz) (J. Vacek).

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*Pisum sativum* and *Oryza sativa* (Mata-Pérez et al., 2017). The endogenous presence of NO<sub>2</sub>OA has been reported in *Brassica napus* (Vollár et al., 2020), *Solanum lycopersicum* (Arruebarrena Di Palma et al., 2020), and *Arabidopsis thaliana* (Di Fino et al., 2020).

The endogenous occurrence of nitro-linolenic acid and NO<sub>2</sub>OA suggests a key role for these molecules in early developmental stages. Nitro-linolenic acid is involved in plant defence processes against abiotic stress conditions through the induction of a network of chaperones and antioxidant systems (Begara-Morales et al., 2021). Based on phytochemical analysis of *Arabidopsis thaliana*, it was shown that phytosterol esters, mono-, di- and triacylglycerides, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol could be the main lipid reservoirs of NO<sub>2</sub>FAs (Aranda-Caño et al., 2022). More details on NO<sub>2</sub>FAs in plants can be found in a very recent review (Aranda-Caño et al., 2026).

The aim of this work is to study for the first time the evidence of NO<sub>2</sub>FAs in microscopic algae and cyanobacteria. For this purpose, we performed targeted LC-HRMS analysis based on synthetically prepared pure NO<sub>2</sub>FA regio/stereoisomeric standards and their non-nitrated (parent) FAs. The semi-quantitative analyses were performed on selected strains of algae and cyanobacteria, specifically the green algae *Desmodesmus communis*, *D. subspicatus*, and *Chlorella* sp.; cyanobacterium *Synechocystis* sp.; and eustigmatophyte *Trachydiscus minutus*.

## 2. Results and discussion

First, we focused on the LC-MS identification of selected parent/precursor saturated and unsaturated C18/C20 FAs and their nitro derivatives, NO<sub>2</sub>FAs, in *Chlorella* sp., *Trachydiscus minutus*, *Synechocystis* sp., *Desmodesmus subspicatus* and *D. communis*. Specifically, stearic (SA), oleic (OA), linoleic (LA) and its conjugated form (cLA), arachidonic (AA) acids, and corresponding NO<sub>2</sub>FAs were profiled. According to the  $t_R$  and exact mass  $[M-H]^- m/z$  values of the pure regio/stereo isomeric standards (Table 1), the pilot findings suggest that selected cyanobacteria and microalgae species constitute both FAs and corresponding NO<sub>2</sub>FAs.

Of the microalgae species examined, *D. communis* and *D. subspicatus* exhibited the greatest amount of non-nitrated FAs. In contrast, the least amount of FAs was found in cyanobacterium *Synechocystis* sp. The highest level of LA along with its conjugated form (cLA) was found in *D. communis* and *D. subspicatus*. The percentage representation of FAs identified throughout the study is clearly demonstrated in Fig. 1A. The concentration of individual FAs was calculated using the single-point calibration method, where semi-quantitative data for C18 FAs-rich *D. communis* are as follows: cLA = 4.49, LA = 27.04, OA = 3.29 and SA = 1.30 mg/g FW. With C20 AA, a concentration of 1.61 mg/g FW was found in *T. minutus*. Based on the already reported studies, commonly observed FAs in *Chlorella* sp. include palmitic acid (C16:0), OA, LA, and  $\alpha$ -linolenic acid (C18:3), their relative amounts vary depending on the specific strain and environmental conditions for their growth (Pantami et al., 2020; Yasmin Anum et al., 2011; Zhang et al., 2014). For *Desmodesmus* sp., 5.43%  $\pm$  0.41% of lipids were obtained on an ash-free dry weight basis with FAs amounting to between 23.62–38.02% of the total

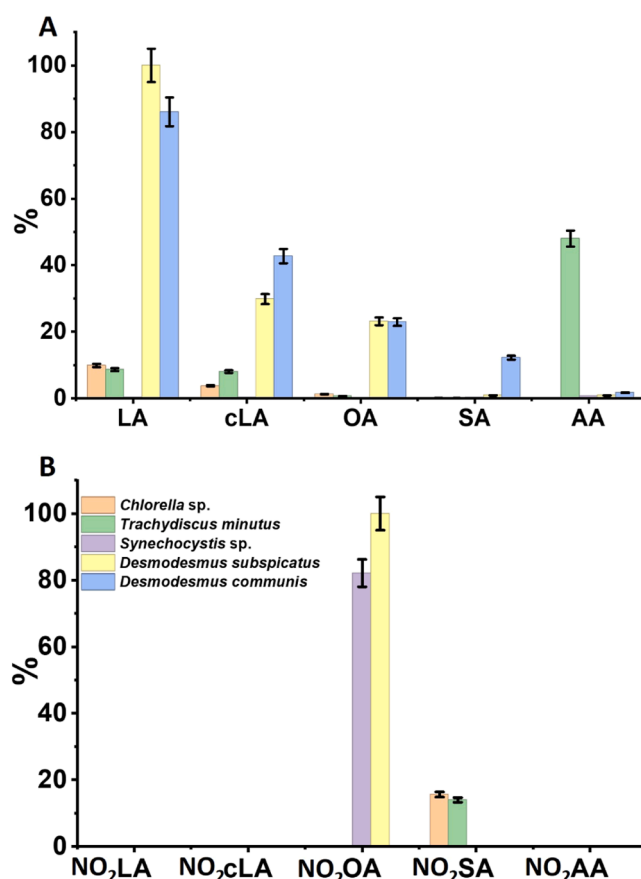


Fig. 1. Percentage representation of individual (A) FAs and (B) NO<sub>2</sub>FAs identified throughout the study. 100% = 260858.2 cps (for LA in panel A) and 173.7 cps (for NO<sub>2</sub>OA in panel B). Data are means  $\pm$  SD of three experiments. Semi-quantitative data can be found in the main text.

lipid pool (Viegas et al., 2020). The most abundant in *Desmodesmus* sp. were found to be palmitic acid, OA, LA and  $\alpha$ -linolenic acid (Solovchenko et al., 2015). The FAs we identified here are therefore consistent with what has been previously documented by other authors in our strains of interest.

The findings from our study reveal the existence of NO<sub>2</sub>FAs in selected strains of algae and cyanobacteria. The percentage representation of identified NO<sub>2</sub>FAs can be found in Fig. 1B. Fig. 2A shows the LC-MS records of NO<sub>2</sub>OA in *D. subspicatus* and *Synechocystis* sp., including a chromatogram and MS pattern for the 10-NO<sub>2</sub>OA standard. Similarly, chromatographic records of NO<sub>2</sub>SA in *Chlorella* sp. and *Trachydiscus minutus* are shown in Fig. 2B. The presence of double peaks can be logically ascribed to more than one isomer existing in the biological samples. The levels of NO<sub>2</sub>OA (820 and 51 ng/g FW for *D. subspicatus* and *Synechocystis* sp.) and NO<sub>2</sub>SA (79 and 65 ng/g FW for *Chlorella* sp.

Table 1

LC-MS retention time ( $t_R$ ) and  $[M-H]^- m/z$  values and other parameters for FAs and NO<sub>2</sub>FAs. For details on chemical standards, see sec. 3.1. For NO<sub>2</sub>OA, 10-NO<sub>2</sub>-isomer is presented. LODs = 3S/N are shown for NO<sub>2</sub>FAs.

| Fatty acid | Non-nitrated |          |                                                |     | Nitrated |          |                                                 |                       |            |
|------------|--------------|----------|------------------------------------------------|-----|----------|----------|-------------------------------------------------|-----------------------|------------|
|            | $t_R$        | $m/z^a$  | Elemental comp.                                | ppm | $t_R$    | $m/z^a$  | Elemental comp.                                 | ppm                   | LOD (ng/g) |
| LA         | 10.15        | 279.2334 | C <sub>18</sub> H <sub>32</sub> O <sub>2</sub> | 3.6 | 8.91     | 324.2184 | C <sub>18</sub> H <sub>31</sub> NO <sub>4</sub> | 2.8                   | 0.82       |
| cLA        | 10.26        | 279.2340 | C <sub>18</sub> H <sub>32</sub> O <sub>2</sub> | 5.7 | 9.09     | 324.2193 | C <sub>18</sub> H <sub>31</sub> NO <sub>4</sub> | 5.6                   | 3.08       |
| OA         | 10.87        | 281.2489 | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub> | 2.8 | 9.48     | 326.2337 | C <sub>18</sub> H <sub>33</sub> NO <sub>4</sub> | 1.8/2.8 <sup>b</sup>  | 2.75       |
| SA         | 11.84        | 283.2647 | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub> | 3.5 | 9.69     | 328.2498 | C <sub>18</sub> H <sub>35</sub> NO <sub>4</sub> | 3.0/-8.5 <sup>b</sup> | 1.41       |
| AA         | 9.96         | 303.2340 | C <sub>20</sub> H <sub>32</sub> O <sub>2</sub> | 5.3 | 8.84     | 348.2185 | C <sub>20</sub> H <sub>31</sub> NO <sub>4</sub> | 2.9                   | 0.29       |

<sup>a</sup> accurate mass observed in analysed solutions of the standards

<sup>b</sup> mass measurement error (mass accuracy, ppm) in the standard/in the sample

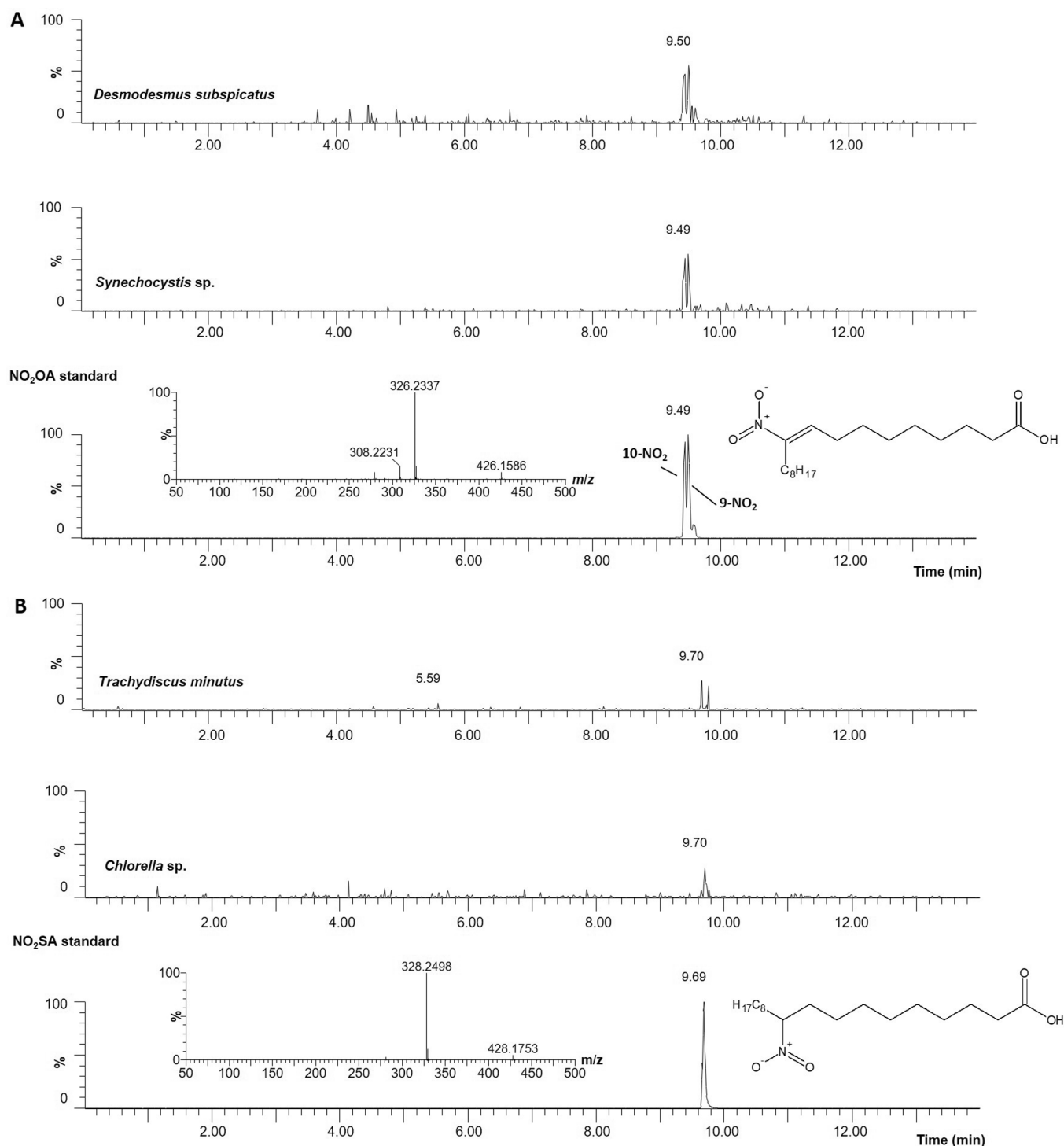


**Fig. 2.** LC-MS records of (A) NO<sub>2</sub>OA and (B) NO<sub>2</sub>SA in selected strains (upper part) and their standards (6  $\mu$ M 10-NO<sub>2</sub>SA and 13.7  $\mu$ M 9/10-NO<sub>2</sub>OA as equimolar mixture), in lower part of the panels, including MS spectra. For other LC-MS identification details, see [Section 2](#) in [Supplementary Information](#).

and *T. minutus*) were more than 1000 times lower than the level of non-nitrated parent FAs, see above. In all analyses, the detected levels of NO<sub>2</sub>FAs were significantly higher than the established limits of detection (LODs), as demonstrated in [Table 1](#). Polyunsaturated (*bis*-allylic) and conjugated NO<sub>2</sub>FAs were not identified in the selected strains, which could be connected with their higher reactivity and rapid degradation kinetics ([Grippio et al., 2021](#); [Liška et al., 2025](#); [Vacek et al., 2022](#)) (during harvesting and sample preparation or manipulation) compared to monounsaturated NO<sub>2</sub>OA or saturated NO<sub>2</sub>SA.

NO<sub>2</sub>FAs can be formed when nitric oxide (NO), or its metabolites and

other reactive nitrogen species, coexist and interact with FAs in algal and cyanobacterial cells. This endogenous production process can lead to the formation of various nitro derivatives, which have different biological activities and implications in signalling pathways. The enzyme machinery is primarily responsible for the production of NO. Besides nitric oxide synthase, nitrate reductase was found to be involved in NO production in various photosynthetic organisms, including algae ([Kumar et al., 2015](#)). Thus, both precursors, NO and FAs, exist in algal biomass to form NO<sub>2</sub>FAs, which we have clearly demonstrated using LC-MS in this study.

In the future, it would be good to focus on profiling NO<sub>2</sub>FAs in a broader panel of algae and cyanobacteria and perform a fully quantitative LC-MS study. At the same time, it would be interesting to verify whether the production of NO<sub>2</sub>FAs in algae and cyanobacteria is associated with the same or similar physiological or stress conditions as have been described for higher plants (Aranda-Caño et al., 2026). Comparative studies of freshwater and marine micro- and macroscopic algae could also provide new insights into the importance of NO<sub>2</sub>FAs for their survival, stress response or growth regulation and development. In addition, experiments with the treatment of algae/cyanobacteria with selected NO<sub>2</sub>FAs, whose cells do not normally produce them endogenously, could contribute to the elucidation of the mechanism of their action, similar to what has been done in plant experiments (Arruebarrena Di Palma et al., 2020). Also, the mechanism for the formation and biological action of saturated NO<sub>2</sub>SA should be clarified, because presence of double-bond is crucial for both (bio)synthesis and electrophile-nucleophile interactions of NO<sub>2</sub>FAs. In this sense, we assume that NO<sub>2</sub>SA could be an inactive metabolic reduction product of NO<sub>2</sub>OA, which would be in accordance with the previously reported data (Vitturi et al., 2013). The data on biological activity of NO<sub>2</sub>SA has not been reported yet.

### 3. Material and methods

#### 3.1. Chemicals

Non-nitrated fatty acids, stearic acid (SA), oleic acid (OA), linoleic acid (LA), conjugated linoleic acid (cLA), and arachidonic acid (AA) were purchased from Merck (Darmstadt, Germany) and used without further purification. All other chemicals were of analytical grade and used as received. NO<sub>2</sub>FAs were synthesized according to our previously published regio- and stereoselective protocols, which combine the Henry reaction with a base-promoted  $\beta$ -elimination step (Chrenko et al., 2025). Using this approach, we prepared 10-nitro-stearic acid (NO<sub>2</sub>SA), nitro-oleic acid (NO<sub>2</sub>OA, both 10- and 9-NO<sub>2</sub> isomers), 10-nitro-linoleic acid (NO<sub>2</sub>LA), and 9/12-nitro-conjugated linoleic acid (NO<sub>2</sub>cLA), each obtained with > 99% regio- and stereochemical purity as confirmed by <sup>1</sup>H and <sup>13</sup>C{<sup>1</sup>H} NMR spectroscopy, and > 98% chemical purity as determined by HPLC analysis. Additionally, 14-nitro-arachidonic acid (NO<sub>2</sub>AA) was synthesized as a single (5Z,8Z,11Z,14E)-14-nitro isomer with > 99% regio- and stereochemical purity, according to a modified procedure (Chrenko et al., 2025), and exhibited > 98% chemical purity as determined by HPLC; for more details see (Zatloukalová et al., 2026).

#### 3.2. Algae cultivation and biomass preparation

Algal stains were obtained from Culture Collection of Autotrophic organisms - CCALA (Institute of Botany, Trebon, Czech republic) and local strain collection of the Institute of Microbiology, Centre Algatech. The algae strains *Chlorella* sp. R-117, *Trachydiscus minutus* CCALA 931, *Synechocystis* sp. PCC 6803, *Desmodesmus subspicatus* CCALA 467 and *Desmodesmus communis* CCALA 463 were cultivated in BG-11 medium using 500 mL glass tubes aerated with air enriched with 1.5% CO<sub>2</sub> at a temperature of 25 °C and continuous artificial illumination of 200  $\mu$ E. The fresh weight (FW) biomass was harvested by centrifugation at 4000 $\times$  g and stored at -80 °C.

#### 3.3. Samples preparation for UPLC/MS<sup>E</sup> analysis

The biomass was dissolved in methanol (10 mg FW/mL) and vortexed for 10 min at 4 °C (Vortex Reax top, Heidolph Scientific Products GmbH, Germany). The samples were further sonicated under the following conditions: 10 pulses of 80% with amplitude 0.5 at 4 °C (UP 200 s, dr. Heischer, GmbH, Germany). The process was performed twice in total. Finally, the samples were centrifuged (Centrifuge MiniSpin, Eppendorf, Germany) at 4 °C and the supernatants were directly

analysed.

#### 3.4. UPLC/MS<sup>E</sup> conditions

The chromatographic separation was performed using an Acquity UPLC system (Waters, Milford, USA) consisting of a binary solvent manager, sample manager, column manager and photodiode array detector. The separation process was done in an Acquity UPLC BEH C18 column, 1.7  $\mu$ m (2.1  $\times$  100 mm) at 30 °C using a gradient elution of mobile phase that consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile delivered at a flow rate of 0.4 mL/min. The analysis time was set to 14 min and the injection volume was 5  $\mu$ L. The gradient profile was set as follows: 0 min 10% B, 10 min 95% B, 11 min 95% B, 13 min 10% B.

A Waters Synapt G2-S Mass Spectrometer (Waters Corp., Manchester, UK) was connected to the UPLC system via electrospray ionization. The ESI source operated in negative ionization mode with the capillary voltage at -2.1 kV and the sampling cone at 25 V. The source temperature and the desolvation temperature were adjusted to 120 °C and 320 °C, respectively. The cone and desolvation gas flows were set to 150 L.h<sup>-1</sup> and 600 L.h<sup>-1</sup>, respectively. Data was acquired from 50 to 1200 Da and processed using the software Masslynx<sup>TM</sup> V4.1 (Waters, Milford, MA, USA). The mass spectrometer was calibrated across the same mass range using a solution of sodium formate in acetonitrile. Data were automatically centroided and mass corrected during acquisition using an external reference leucine-enkephalin (20  $\mu$ g.L<sup>-1</sup> in mixture of water:acetonitrile:formic acid (100:100:0.2), flow rate of 10 mL.min<sup>-1</sup>).

Two interleaved scan functions (MS<sup>E</sup> experiments), which enables the simultaneous acquisition of both low collision energy (CE) and high collision energy mass spectra from a single experiment, were used for data acquisition. MS spectra were acquired at 4 eV for trap CE and at 2 eV for transfer CE (Function 1). Function 2 (high CE) was operated under the following conditions: the trap CE was set to 4 eV and the transfer lenses were ramping CE in the range of 10–30 eV. The scan time of each event was set to 0.2 s. The analyser was working in resolution mode. Post-acquisition processing of the data was performed using Metabolynx V4.1 (Waters Corp., Milford, MA, USA).

Compound identification was based on the combination of accurate mass measurement (mass deviation  $\leq$ 10 ppm, including at low signal intensities) and precise matching of the chromatographic retention time to the corresponding authentic standard, rather than on precursor *m/z* alone; only peaks eluting at the same retention time as the respective standards were considered. For isomeric compounds, individual isomers were not distinguished, and their concentrations were therefore quantified as the combined sum, analogously to the corresponding standard. Details on procedure for semi-quantitative LC-MS is described in Section 1 in Supplementary Information.

### 4. Conclusions

Utilizing a highly targeted LC-MS approach, specifically employing synthesized ultrapure standards of NO<sub>2</sub>FA regio/stereoisomers, we were able to demonstrate the production of NO<sub>2</sub>OA in the strains of *Synechocystis* sp. and *D. subspicatus*, in addition to also revealing the presence of NO<sub>2</sub>SA in the species *Chlorella* sp. and *T. minutus* for the first time. Remarkably, the concentrations of the NO<sub>2</sub>OA and NO<sub>2</sub>SA (at ng level per gram of FW biomass) were found to be more than 1000 times lower than the concentrations observed for the non-nitrated parent FAs. These findings indicate that various freshwater algae and cyanobacteria possess the remarkable capability to produce both saturated and unsaturated forms of NO<sub>2</sub>FAs.

#### CRedit authorship contribution statement

**Barbora Papoušková:** Writing – original draft, Methodology. **Daniel Chrenko:** Writing – original draft, Methodology. **Pavel**

**Hrouzek:** Writing – original draft, Methodology. **Martina Zatloukalová:** Writing – original draft, Methodology. **Jirí Pospíšil:** Writing – original draft, Project administration, Methodology. **Jan Vacek:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.phytol.2026.104252](https://doi.org/10.1016/j.phytol.2026.104252).

## Data availability

Data will be made available on request.

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