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Evaluating the relevance of species sorting and physiological plasticity of phytoplankton communities grown in a multifactor environment

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Abstract

1. The two important mechanisms influencing the response of phytoplankton communities to alterations of abiotic factors in their environment are difficult to distinguish: species sorting resulting from a change in interspecific competitive pressure, and phenotypic plasticity (here explicitly physiological plasticity i.e. species-specific physiological adjustment). A shift in species composition as well as physiological adjustments in species can lead to changes in fatty acid composition that determine the food quality for zooplankton consumers.
2. We used phytoplankton communities consisting of five species and exposed them to two different light intensities, two light conditions (constant and variable), and two levels of phosphorus supply. Changes in fatty acid and species composition were analyzed. We compared community pairs differing in one factor by calculating the Bray-Curtis similarity index for the composition of both variables. Comparing the Bray-Curtis similarity index of the species composition with the index of the fatty acid composition was used to estimate the effects of species sorting and physiological plasticity.
3. Changes in nutrient supply influenced fatty acid responses based on species sorting and physiological plasticity the most. On one hand, the relevance of physiological plasticity was highest at cultivation in different nutrient supplies but the same light environment. Conversely with low nutrients species sorting appeared to dominate the response to changes in light, while at high nutrients physiological plasticity appeared to influence the response. Overall, under low phosphorus supply the communities showed a lower total fatty acid content per carbon and had increased proportions of saturated and monounsaturated fatty acids. Instead, communities in low light produced more of eicosapentaenoic acid.
4. Our results suggest that the relevance of species sorting and physiological plasticity in shaping the community response highly depends on the environmental factors that influence the system. Nutrient supply had the largest effect, while light had more limited conditional effects. However, all of these factors are important in shaping the food quality of the phytoplankton community for higher trophic levels.

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KEYWORDS
fatty acid composition, light intensity, light variability, nutrient supply, resource competition

1 | INTRODUCTION

Phytoplankton communities are a combination of various species with different traits that can each react in different ways to changes in their environment. In general, it is difficult to distinguish between the two underlying mechanisms responsible for these responses in phytoplankton communities. In the first mechanism, shifts in abiotic factors can cause changes in the fundamental niches and competition leading to species sorting and therefore changes in the community composition. In the second, individual organisms and species adjust their morphology or physiology as a reflection of altered abiotic or biotic environmental conditions. These reactions are known as phenotypic or physiological plasticity. Thus, environmental factors simultaneously alter community structure as well as the physiological response of species in the community, making these two mechanisms difficult to separate (Hill et al., 2011; Maloufi et al., 2016). An important responsive physiological marker in aquatic food webs is the fatty acid composition, which is influenced by both species sorting (Galloway & Winder, 2015) and physiological plasticity (Piepho et al., 2012). On one hand, species sorting might be the direct consequence of species competing for limiting resources, for example, with some species becoming dominant and others being outcompeted. As there is substantial variation among the major taxonomic clades and species in important traits such as size, pigment and lipid composition (Ahlgren et al., 1992; Finkel et al., 2016; Roy et al., 2011), the dominance of certain species tends to determine the overall composition of fatty acids (Hartwich et al., 2012; Marzetz et al., 2017). Therefore, a change in the community species composition can have a large influence on the fatty acid composition, which is crucial for higher trophic levels, as polyunsaturated fatty acids (PUFA) play important roles in the cells of all eukaryotic organisms and influence growth and reproduction of herbivorous zooplankton (Ahlgren et al., 2005; Anthony et al., 2009; Lukas & Wacker, 2014; Müller-Navarra et al., 2000; Rossoll et al., 2012; Wacker & Elert, 2001). On the other hand, each species in a community can specifically react to certain conditions or environmental changes, by changing its physiology to adjust to new conditions. This physiological plasticity can also change the overall composition of fatty acids in a phytoplankton community (Wacker et al., 2015). For instance, in ecological adaptation of microalgae much higher levels of PUFA were found in response to certain conditions (Blanchemain & Grizeau, 1996; Klyachko-Gurvich et al., 1999). Consequently, both mechanisms, species sorting and physiological plasticity, are likely to play important roles in determining the effects of environmental changes such as light or temperature (Teurlincx et al., 2017; Yvon-Durocher et al., 2017). Generally both mechanisms appear to be important and are not independent from each other, especially when environmental fluctuations influence the system. Environmental fluctuations change the availability of certain resources (e.g. nutrients and light) and may cause physiological plasticity. This influences each species differently and may alter competitive interactions thus changing the competitive outcome (Yang & Jin, 2008). In turn this results in species sorting, and thus a changed community composition (Litchman et al., 2012; Stomp et al., 2008). Furthermore, it has been suggested that primarily the phytoplankton community composition rather than environmental factors are determining basal food quality in aquatic habitats (Galloway & Winder, 2015).

To disentangle the effects of species sorting and physiological plasticity we propose to use a similarity index applied to two different response variables in communities experiencing different environmental conditions. One index is calculated on the basis of the species composition and the other on the basis of the fatty acid composition. By opposing the two indices, the relative effects of species sorting and physiological plasticity on the community fatty acid composition can be estimated (Figure 1a). Linking these two similarity indices by plotting them against each other can result in three possible scenarios (Figure 1a): (a) we observe a simultaneous change in the species composition and the fatty acid composition that is exactly proportional. Thus, both similarity indices co-vary and shift along the bisecting line, i.e. the 1:1 line between both indices. This should indicate the sole occurrence of species sorting, because a change of the species composition is then directly affecting the fatty acid composition. Indices that are varying independently describe communities that are either very dissimilar in their species composition, but quite similar in their fatty acid composition or vice versa, as a result of physiological plasticity. In the first case (b) indices should deviate to the left side of the 1:1 line. This might indicate that species adjusted their fatty acid profile according to the environmental factors. In the other case (c) varying indices describe that the species composition of communities from different environments is relatively similar, the species could have adjusted to the environmental conditions showing strongly dissimilar fatty acid compositions. Then the indices would shift to the right hand side of the 1:1 line.

Many environmental factors that influence these two mechanisms have been examined for their effect on the fatty acid composition of phytoplankton species. The availability of nutrients together with other important environmental factors such as temperature and light showed interactive effects on the primary producers' quantity and their nutritional quality for the consumers (Schälicke et al., 2019; Sterner et al., 1997). While a low phosphorus (P) supply results in higher proportions of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA, Hill et al., 2011; Piepho et al., 2012), the PUFA content increases with lower irradiances (Blanchemain & Grizeau, 1996; González et al., 2019; Hill et al., 2011; Piepho et al., 2012). The latter possibly enhances the functioning

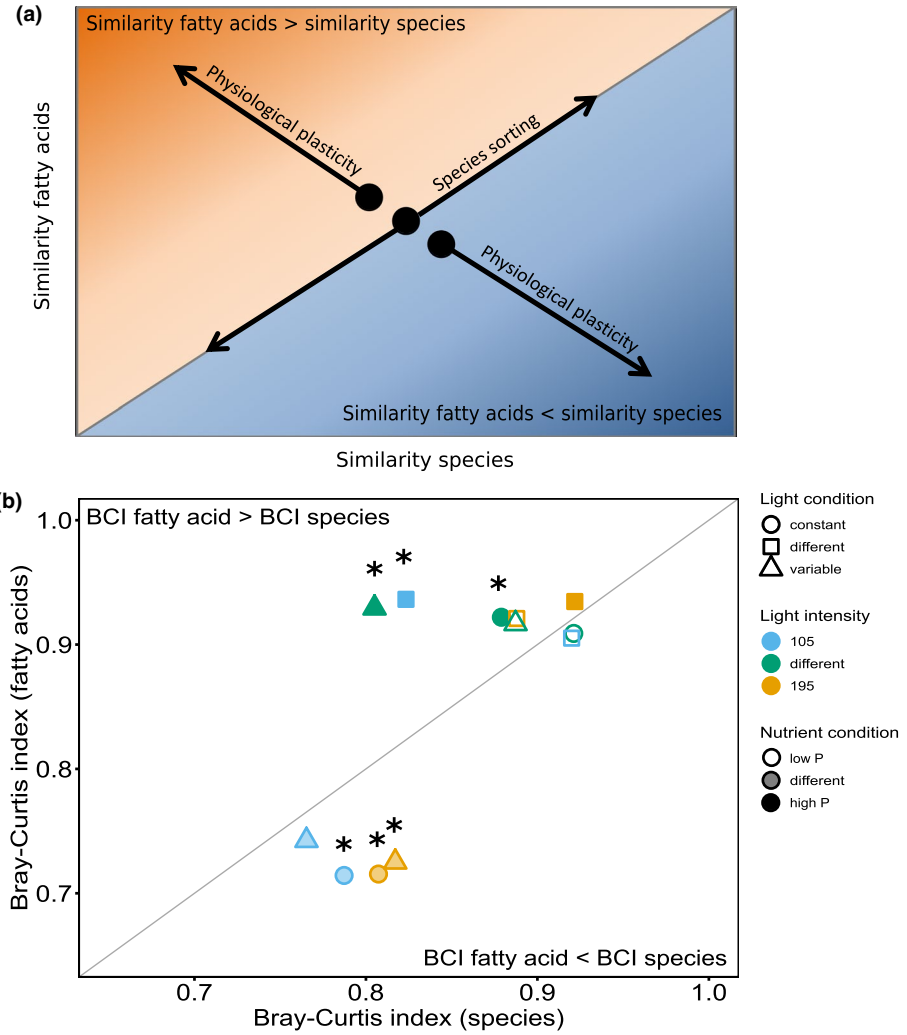

FIGURE 1 Bray-Curtis similarity indices (BCI) for the relative species composition (*x*-axis) compared with the relative fatty acid composition (*y*-axis). (a) Schematic showing that co-variation of both indices (1:1 line) indicate species sorting and any deviation from the 1:1 line shows the increasing relevance of physiological plasticity. (b) Bray-Curtis indices of the experimental communities. For the Bray-Curtis similarity index specific communities were compared pairwise, where always only one experimental factor differed (square symbol, green color, and intermediate shade) e.g. two communities where cultured in a constant 195 μmol photons·m^{-2}·s^{-1} light intensity (yellow circle), but one was cultured in high and the other in low P-supply, thus differed in their nutrient condition (half shaded yellow). Asterisks show significant differences between Bray-Curtis indices of species and fatty acids (Table 1). Data points above the grey diagonal line are more similar in their fatty acid composition than their species composition, whereas data points below are more similar in their species composition than their fatty acid composition. Note: for clarity *SD* are not shown and can be found in Table 1

of the photosynthetic membranes (Blanchemain & Grizeau, 1996; Klyachko-Gurvich et al., 1999). At high light intensities, excess carbon is often accumulated in storage lipids that are rich in SFA and MUFA (Guschina & Harwood, 2009; Piepho et al., 2012). An interaction of light intensity and nutrient supply has been demonstrated (González et al., 2019; Hill et al., 2011; Piepho et al., 2012), though the effect is different for different nutrients. Nitrogen depletion increased the PUFA content in single species experiments in diatoms (González et al., 2019), whereas a high phosphorous supply increased the PUFA content of natural stream periphyton (Hill et al., 2011). While there were several studies conducted on how phytoplankton fatty acid composition is influenced by nutrient availability, light intensity, and interactions between both, the effects of light variability although highly important in nature are largely unknown.

Here, we aim to unravel the mechanisms of species sorting and physiological plasticity while investigating the reaction of a diverse assemblage of phytoplankton to three important variables in natural waters: nutrient availability, light intensity, and light variability. The response of the phytoplankton communities to these potentially limiting or stressful environmental factors could have large effects on the performance of consumers. We assembled five phytoplankton species and led them grow together in a community. This allows for interactions among the species and can e.g. result in a more beneficial fatty acid composition for consumers (Wacker et al., 2015).

TABLE 1 Pairwise community comparison for the Bray-Curtis similarity index using either the proportional species composition (Index species) ± *SD* and the proportional fatty acid composition (Index FA) ± *SD*

Com A	Com B	Differed exp. factor	Index species	Index FA	*t*	*p*
HighP.105.**con**	HighP.105.**var**	Light condition	0.82 ± 0.07	0.94 ± 0.01	−4.75	**<0.0063**
HighP.**105**.con	HighP.**195**.con	Light intensity	0.88 ± 0.02	0.92 ± 0.03	−3.55	**<0.0071**
HighP.105.con	**LowP**.105.con	Nutrients	0.79 ± 0.04	0.71 ± 0.02	4.98	**<0.0046**
HighP.**105**.var	HighP.**195**.var	Light intensity	0.81 ± 0.03	0.93 ± 0.01	−10.55	**<0.0042**
HighP.105.var	**LowP**.105.var	Nutrients	0.77 ± 0.08	0.74 ± 0.04	0.75	0.4716
HighP.195.**con**	HighP.195.**var**	Light condition	0.92 ± 0.03	0.93 ± 0.03	−0.81	0.4320
HighP.195.con	**LowP**.195.con	Nutrients	0.81 ± 0.05	0.72 ± 0.03	4.38	**<0.005**
HighP.195.var	**LowP**.195.var	Nutrients	0.82 ± 0.04	0.73 ± 0.05	4.54	**<0.005**
LowP.105.**con**	LowP.105.**var**	Light condition	0.92 ± 0.05	0.91 ± 0.01	0.79	0.4513
LowP.**105**.con	LowP.**195**.con	Light intensity	0.92 ± 0.06	0.91 ± 0.01	0.64	0.5399
LowP.**105**.var	LowP.**195**.var	Light intensity	0.89 ± 0.06	0.92 ± 0.04	−1.29	0.2182
LowP.195.**con**	LowP.195.**var**	Light condition	0.89 ± 0.06	0.92 ± 0.02	−1.59	0.1423

Notes: For the Bray-Curtis similarity index specific communities were compared pairwise (Com A vs. Com B), in which always only one experimental factor differed ("Differed exp. factor", additionally shown in bold in Com A and Com B where var = variable light condition, con = constant light condition, and the number represents the light intensity in μmol photons·m^{-2}·s^{-1}). The two similarity indices were then compared with each other using a two-sample *t*-test. Significant *p*-values are shown in bold numbers ($n = 9$) after a sequential Bonferroni correction of α.

We hypothesize that the relative importance of species sorting and physiological plasticity depends on environmental factors such as nutrient supply, light intensity and variability to which the phytoplankton responds. Physiological plasticity might in particular be at play as a response to altered light intensity and light condition. The mechanism of competitive replacement of species might be stronger regarding changes in the nutrient supply. As a physiological response, we expect that reducing light intensity and increasing nutrient supply could increase the proportions of long-chained polyunsaturated fatty acids in the cultures.

To investigate the relative effects of both mechanisms and the general effect of environmental factors on the fatty acid composition we ran laboratory experiments with communities consisting of five phytoplankton species originating from different taxonomic (and functional) groups. We evaluated the phytoplankton community fatty acid profiles and species composition based on their exposure to different conditions of three factors: constant or variable light, differing mean light intensities, and different nutrient supplies. To estimate the relative contributions of species sorting and physiological plasticity on the community response regarding the changes in environmental factors we applied the Bray-Curtis similarity index for both species and fatty acid compositions among communities with different environmental factors.

2 | MATERIALS AND METHODS

2.1 | Phytoplankton cultures

Five freshwater phytoplankton species were used in this experiment. They were chosen based on taxonomic affiliation and specific traits such as different fatty acid composition.

In detail we used the cyanophyte *Synechococcus elongatus* Nägeli (Syn, SAG 89.79), the chlorophyte *Tetradesmus obliquus* (Turpin) M.J. Wynne (Tet, SAG 276-3a, formerly named *Acutodesmus obliquus* (Turpin) Hegewald et Hanagata), the diatom *Stephanodiscus hantzschii* Grunow (Ste, University of Constance), the cryptophyte *Cryptomonas ovata* Ehrenberg (Cry, SAG 979–3), and the eustigmatophyte *Nannochloropsis limnetica* Krienitz, Hepperle, Stich, Weiler, (Nan, SAG 18.99).

Every phytoplankton species was pre-cultured axenically either in high P-supply (50 μmol P/L, in the form of K_2HPO_4) or low P-supply (5 μmol P/L) modified Woods Hole MBL medium with a nitrogen supply of 1,000 μmol N/L (in the form of $NaNO_3$) (Nichols, 1973). To avoid a limitation by potassium in the cultures with a low P-supply, the final concentration of potassium was adjusted to 100 μmol/L using potassium chloride.

2.2 | Experimental set up

The different species pre-cultures were combined into a community and grown under different P and light conditions. The optical density of the pre-cultures were measured and the carbon concentration estimated with a previously determined carbon light equation. These equations were determined for each species by establishing a species culture with a certain extinction value at 800 nm (OD_{800}) and followed carbon analysis (see chemical analysis). Aliquots of the pre-cultures were used to inoculate the experimental phytoplankton communities with equal carbon shares of each species. A total biomass concentration of 1.67 and 0.67 mg C/L was established for cultures with

high and low P-supply, respectively. All experimental phytoplankton cultures were grown semi-continuously (0.067 d^{-1}) in triplicate, at 20°C in 500 ml Erlenmeyer flasks filled with a total volume of 300 ml.

The constant light intensities with a regular 12/12 hr light/dark cycle were set to 105 or 195 µmol photons·m^{-2}·s^{-1} using different numbers of fluorescent tubes (FLUORA L30W / 77 and LUMILUX L30W/830, warm white, Osram AG, München, Germany) with different distances to the flasks. Additional darkening foil (neutral density foil filters, Lee Filters, Hampshire, England) was used if necessary. The light intensity was verified with a spherical light sensor (LI-1400 data logger; LI-COR Environmental GmbH, Bad Homburg, Germany, equipped with a 4π quantum sensor). Additionally, two variable light conditions were set up, in which phytoplankton communities were irradiated with the same average light intensities as communities exposed to constant light intensities. They were exposed to 3 hr lower light intensity, 6 hr higher light intensity, and again 3 hr of the lower light intensity, with the high/low shifted by 90 µmol photons·m^{-2}·s^{-1} plus/minus from the mean light intensity (Supplementary Material 1, Figure 1). This cycle was followed by 12 hr darkness. Consequently, the variability range of the light intensity from low to high light in both variable light treatments was the same, but the mean light intensity was different. The cultures with a high P-supply were harvested after 6 days, whereas the cultures with a low P-supply after 9 days of growth. As all the cultures received fresh medium and thus nutrients daily the different harvesting times avoided a large change in the treatment light intensity due to high cell densities in high P supply cultures, whilst low P-supply cultures had more time to obtain sufficient biomass with similar optical density for harvesting. Consequently, the low P supply cultures grew 3 more days to reach a similar optical density as the high P-supply cultures.

2.3 | Community composition

Phytoplankton species distribution in the communities, cell numbers, and biovolumes of the phytoplankton cultures were determined. Samples were fixed with Lugol's iodine solution and phytoplankton cells were counted using an inverted light microscope (Thalheim Spezial Optik, Pulsnitz, Germany). The cyanobacterium was counted by epifluorescence microscopy (Axioskop 2, Carl Zeiss, Jena, Germany) under blue light (excitation: BP 450–490 nm; emission: BP 515–565 nm) after staining with acridin-orange (Merck). Sizes of phytoplankton cells were measured and converted into biovolume according to Hillebrand et al., (1999). The community composition was calculated based on the cell numbers and biovolumes.

2.4 | Chemical analysis

Each phytoplankton culture was analyzed for particulate organic carbon and fatty acids. For carbon analysis, aliquots of the algal suspensions were filtered onto 25 mm precombusted glass fiber filters (GF/F, Whatman, Dassel, Germany), dried at 50°C for 48 hr and analyzed using an elemental analyzer (Euro EA 3000; HEKAtech GmbH). Samples for fatty acid determination were obtained by filtering aliquots of the algal onto 25 mm glass fiber filters (GF/F, Whatman). Filters were immediately frozen in liquid nitrogen, then stored at −80°C. Before extraction filters were transferred in 7 ml of dichloromethane-methanol (2:1 v/v) under a nitrogen atmosphere in glass tubes with Teflon seals. Lipids were extracted and transesterified into fatty acid methyl esters and then identified and quantified by gas chromatography (GC 6890N; Network GC System, Agilent Technologies GmbH) according to Wacker et al. (2016). The total fatty acid content (TFA) was related to the respective carbon concentrations of the phytoplankton culture. Specific fatty acids were related to the TFA content.

2.5 | Similarity index

To estimate the relative contributions of species sorting and physiological plasticity on the community response regarding the tested environmental changes we calculated the Bray-Curtis similarity index (Bray & Curtis, 1957). This index (BCI) uses the species or fatty acid specific proportions (p) of two communities which were exposed to different experimental conditions (*x* and *y*, Equation 1). We calculated this index for both the proportional species and fatty acid composition (List of fatty acids see Supplementary Material 1, Table 1). Generally an index of 1 means the two compared communities are identical, whereas an index of 0 means that they are completely different. The index is affected by proportion and presence/absence of the parameter.

$$\mathrm{BCI} = 1 - 0.5 \sum_{i=1}^{i} \left| p_{xi} - p_{yi} \right| \quad (1)$$

Theoretically, these calculations would generate 36 similarity indices, where eight are comparing identical communities and 28 similarity indices comparing all possible combinations. For further interpretation, we chose only the 12 comparisons between communities changing solely one environmental factor at a time. For example, communities were compared that were both cultivated with low nutrient supply and a mean light intensity of 105 µmol photons·m^{-2}·s^{-1}, but differed in their light condition. This means that one community was cultivated with constant light, whereas the other was cultivated in variable light. This way we were able to (a) evaluate qualitatively the effect of the environmental factor at play, which leads to a deviance from the similarity in the species and fatty acid composition and (b) use different responses in species and fatty acid similarity to identify the underlying mechanism, *i.e.* species sorting or physiological plasticity.

2.6 | Data analyses

The resulting indices of the species composition were compared with the indices of the fatty acid composition using a two-sample

t-test. A sequential Bonferroni correction was performed for the 12 *t*-test results.

The TFA was normalized onto the carbon measured in the samples (μg TFA/mg C), whereas the individual fatty acids or fatty acid groups were given in proportions of TFA.

Effects of nutrient availability (high and low P-supply), average light intensity (105 or 195 μmol photons·m^{-2}·s^{-1}), and light condition (constant or variable) on fatty acid concentrations were tested using three-way ANOVA.

A principal component analysis was carried out using the sum of the SFA, MUFA, PUFA, as well as the MUFA oleic acid (OLA, C18:1n-9), the PUFA linoleic acid (LNA, C18:2n-6), α-linolenic acid (ALA, C18:3n-3), arachidonic acid (ARA, C20:4n-6), and eicosapentaenoic acid (EPA, C20:5n-3) as variables. The single fatty acids which were individually included in the PCA were omitted from the sums of the respective fatty acid groups to avoid redundancy in the analysis.

All calculations and statistical analyses were carried out using the statistical software package R (R Development Core Team, 2010). The Bray-Curtis similarity index was calculated using the vegan R package (version 2.5-6).

3 | RESULTS

3.1 | Bray-Curtis similarity index

The Bray-Curtis similarity index ranged from 0.77 to 0.92 and 0.71 to 0.94 for species and fatty acid composition, respectively (Table 1). The differences in community and fatty acid composition are a result of the changed environmental factors. For instance, does the single values of cultures grown either at high or low P conditions and in variable light conditions with a mean light intensity of 195 μmol photons·m^{-2}·s^{-1} with a species index of 0.82 ± 0.04 and fatty acid index of 0.73 ± 0.05 mean that they differed from each other by 18% and 27%, respectively (Table 1).

In order to disentangle the effects of species sorting and physiological plasticity the relationship between Bray-Curtis indices of the fatty acid and species composition was analyzed. There a deviation from the bisecting line shows that the species composition changed independently (or at least less dependent) from the community fatty acid composition. This was the case when the light intensity among the communities was different, some of their similarity indices deviated from the 1:1 line to the left (Figure 1b, green symbols). This deviation was significant under high phosphorus supply (filled green symbols), both at variable and constant light conditions (green triangle vs. circle, respectively). At low nutrient supply (empty symbols), however, did the similarity indices co-vary i.e. were found along the bisecting line independently if light intensity or condition was different among the communities (Figure 1b). If nutrient supply among the communities was different (Figure 1b, half shaded symbols), the similarity of fatty acids was generally lower, and all light treatments, except the variable, low light (half transparent triangle) led to a deviation of the similarity indices to the right of the 1:1 line. Interestingly, if light condition was different, i.e. variable versus constant light, similarity indices deviated from the 1:1 line at low light and high phosphorus supply, only (Figure 1, blue, filled square).

3.2 | Fatty acid composition

Different light intensities and conditions, as well as nutrient supply lead to different fatty acid compositions. The P-supply appeared to have the strongest effect on the communities and resulted in differences in most fatty acids or fatty acid groups (Figure 2, Table 2).

The content of TFA was about three to four times higher in communities that were supplied with high P concentrations, and there the light did not affect the concentration further (Figure 2, Table 2).

Fatty acid groups and specific fatty acids were normalized to TFA so to represent the proportion of each fatty acid. With high P-supply each of the proportion of SFA and MUFA of TFA were 15%–24%, whereas in low P-supply each of them accounted for up 30 to 35% (Figure 2). When supplied with low P, communities contained about 15% more SFA and MUFA compared to those supplied with high P. Additionally, with increasing light intensity, the proportion of MUFA decreased, whereas the SFA tended to increase (Figure 2, Table 2). The proportion of PUFA showed the opposite pattern, where the cultures with high P-supply had ca. 60% PUFA, which is approximately 20% more than the cultures that were supplied with low P (Figure 2, Table 2).

Fatty acids that are nutritionally highly relevant for zooplankton consumers such as essential fatty acids, and their potential precursor OLA are closer addressed here due to their importance. Reactions of specific fatty acids varied, and all except EPA were affected by P-supply as single factor in some way (Figure 2, Table 2). Low P-supply and light variability lead to an increase of OLA to ca. 5% compared to 3.5% at high P-supply (Figure 2, Table 2). Light intensity influenced the outcome of light variability resulting in a higher proportion of OLA when the light intensity was lower (Figure 2, Table 2, interaction of intensity and condition). The two C18 fatty acids LNA and ALA reacted in a comparable manner (Figure 2, Table 2), with an approximate doubling in their proportions, if changed from low to high P-supply (LNA from 3.5% to 5.5% and ALA from 10% to 25%–30%). Additionally the proportion increased, when the cultures were supplied with a higher mean light intensity, resulting in a proportion increase of ca. 1% and 5% for LNA and ALA, respectively (Figure 2, Table 2). The long chained C20-PUFAs ARA and EPA reacted quite differently from each other (Figure 2, Table 2). ARA in communities decreased from ca. 1.6% to only 1% – 1.3% if supplied with high instead of low P (Figure 2, Table 2). Additionally, the light condition influenced the outcome of the P-supply effect (Table 3, interaction of condition and P-supply). There low P and constant light or high P and variable light lead to higher ARA proportions. The ARA proportion was affected by all three factors, as the community grown in high nutrient supply and under low and variable light conditions showed a higher ARA

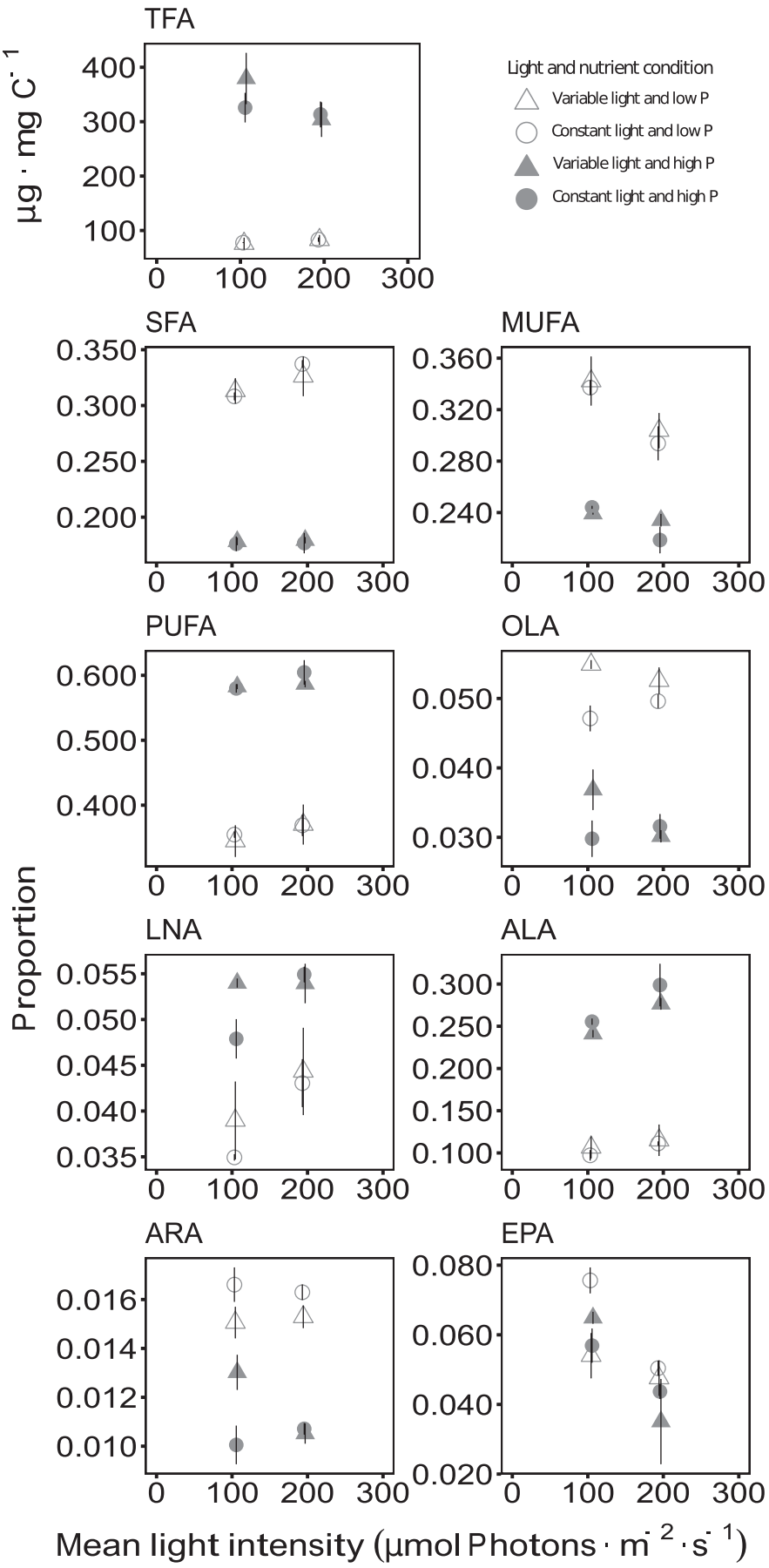

FIGURE 2 Total fatty acid concentrations (TFA, µg fatty acid per mg carbon) and relative amounts to TFA of certain fatty acid groups (SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids), as well as specific, for food quality relevant, fatty acids or its precursors, such as oleic acid (OLA, C18:1n9), linoleic acid (LNA, C18:2n6), ∝-linolenic acid (ALA, C18:3n3), arachidonic acid (ARA, C20:4n6), and eicosapentaenoic acid (EPA, C20:5n3). Communities were either cultivated under variable (triangles) or constant (circles) light conditions. Mean values are shown for high (filled symbols) and low (empty symbols) nutrient supply and are displayed with ± standard error

TABLE 2 ANOVA table for all factors (light intensity, light condition, and nutrient condition) including interactive effects conducted for all response variables (Figure 2). Significant results are shown in bold numbers. Acronyms are the same as in the Figure 2

			TFA[a]		MUFA		SFA		PUFA	
Factor	**dfN**	**dfD**	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Light intensity (LI)	1	16	1.20	0.29	**14.17**	**<0.01**	3.13	0.10	2.07	0.17
Light condition (LC)	1	16	0.37	0.55	0.73	0.41	0.00	0.96	0.26	0.62
Nutrient condition (NC)	1	16	**212.2**	**<0.001**	**130.5**	**<0.001**	**537.2**	**<0.001**	**368.8**	**<0.001**
LI × LC	1	16	0.81	0.38	0.71	0.41	0.40	0.54	0.04	0.85
LI × NC	1	16	2.14	0.16	2.96	0.10	2.65	0.12	0.05	0.82
LC × NC	1	16	0.45	0.51	0.02	0.88	0.19	0.67	0.02	0.90
LI × LC × NC	1	16	0.88	0.36	0.27	0.61	0.44	0.52	0.45	0.51

			OLA		LNA		ALA		ARA		EPA	
Factor	**dfN**	**dfD**	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Light intensity (LI)	1	16	0.82	0.38	**7.21**	**<0.05**	**8.03**	**<0.05**	1.43	0.25	**20.79**	**<0.001**
Light condition (LC)	1	16	**9.60**	**<0.001**	1.87	0.19	0.43	0.52	0.02	0.90	2.36	0.14
Nutrient condition (NC)	1	16	**206.3**	**<0.001**	**42.13**	**<0.001**	**323.3**	**<0.001**	**138.6**	**<0.001**	2.73	0.12
LI × LC	1	16	**6.35**	**<0.05**	1.67	0.22	0.15	0.71	2.65	0.12	0.02	0.90
LI × NC	1	16	0.92	0.35	0.72	0.41	2.48	0.14	1.20	0.29	0.48	0.50
LC × NC	1	16	0.93	0.35	0.00	0.98	1.95	0.18	**11.00**	**<0.01**	2.12	0.17
LI × LC × NC	1	16	0.49	0.50	0.32	0.58	0.00	0.95	**5.29**	**<0.05**	**4.68**	**<0.05**

[a]TFA is given in μg TFA/mg C while the other fatty acids are given in proportions of TFA.

TABLE 3 Summary of the principal component analysis (Figure 3) including the fatty acid groups (SFA, MUFA, PUFA) and the single for food quality relevant fatty acids (OLA, LNA, ALA, ARA, EPA), all relative to TFA, as variables

	PC1	PC2	PC3
sdev	2.49	0.98	0.67
Eigen.val	6.18	0.97	0.46
expl.VAR	77.19	12.07	5.69
SFA	**0.38**	−0.27	0.12
MUFA	**0.37**	0.07	**−0.48**
PUFA	**−0.38**	0.05	0.00
OLA	**0.36**	−0.19	**0.45**
LNA	**−0.36**	−0.17	**0.60**
ALA	**−0.39**	0.07	−0.05
ARA	**0.37**	−0.03	**0.32**
EPA	0.15	**0.92**	**0.30**

Note: the PUFA and MUFA group do not include the single fatty acids. Loadings marked bold and in light and dark grey were considered significantly positively and negativley correlating with the respective PC axis. Acronyms are the same as in Figure 2.

proportion than communities grown in high light regardless of the light condition (Figure 2, Table 2, significant three-way interaction). Unlike all the other fatty acids, EPA did not show an effect to nutrient supply, but a strong reaction to increased light intensity, where the EPA proportion decreased from ca. 5%–8% to 4%–5% (Figure 2, Table 2). Additionally, EPA proportions of communities grown under low P-supply and variable light were less affected by light intensity than in all other communities (Figure 2, Table 2, significant three-way interaction).

3.3 | Principle component analysis (PCA)

The PC 1 explained 77.2% of the variation in the data and separated the communities depending on the P-supply (Figure 3a, b, Table 3). Communities grown under low P-supply showed positive scores on PC1, associated with high proportion of SFA, MUFA, ARA, and OLA. Communities grown with high P-supply showed negative scores on PC1, which was associated with high proportions of PUFA, LNA, and ALA. The PC2 explained 12.1% of the variation in the data, separated the communities roughly by mean LI and EPA correlated positively, showing an increased proportion at the lower mean light intensity (Figure 3a, Table 3). The PC3 explained 5.7% of the variation in the data (Table 3), and separated communities grown at high P-supply and low light intensities depending on their light condition (Figure 3b).

4 | DISCUSSION

We demonstrate here that (a) simultaneously using the similarity index on the species and fatty acid composition the mechanisms of

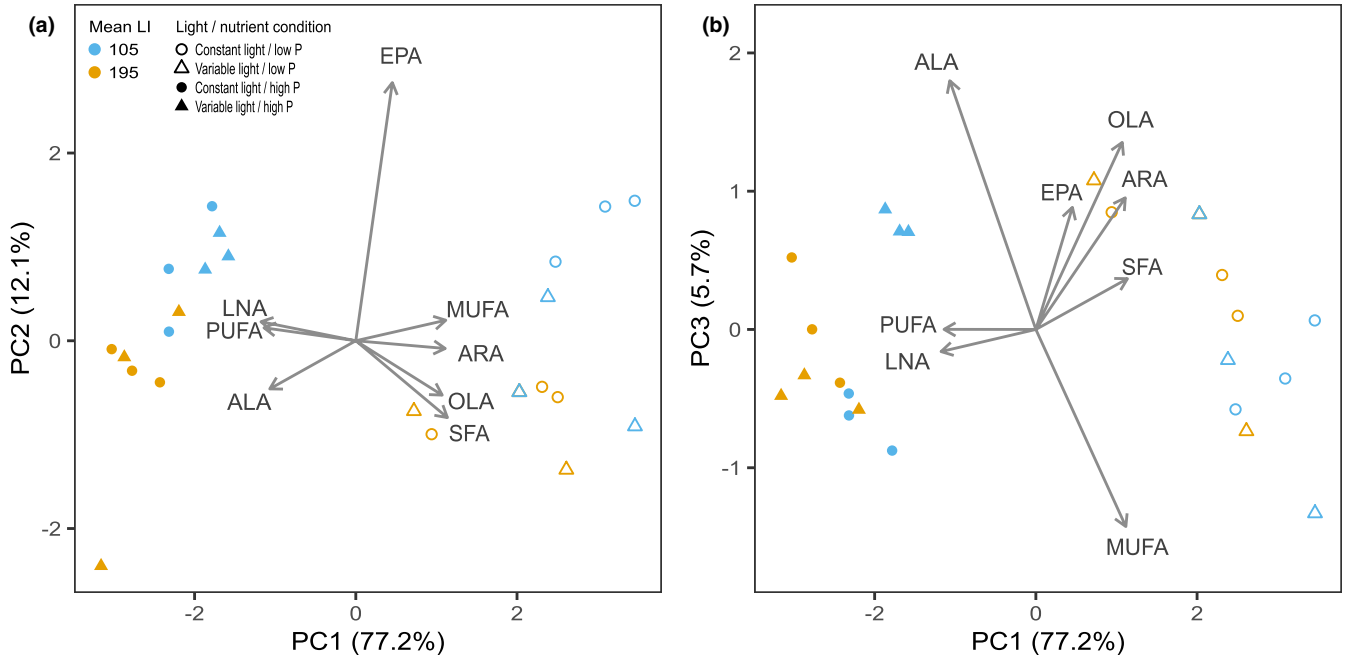

FIGURE 3 One principal component analysis (a, b) including single for animal nutrition relevant fatty acids (OLA, LNA, ALA, ARA, EPA) always as separate variables, and all other fatty acids were included in the same PCA as sums of specific fatty acid groups (SFA, MUFA, PUFA). All fatty acids are given relative to TFA (List of fatty acids see Supplementary Material 1, Table 1), and PUFA as well as MUFA do not include the above mentioned single fatty acids. Communities were grown at different light intensities (colors), at constant (circle) or variable (triangle) light, and high (filled symbols) or low (empty symbols) nutrient supply. Note: the variable light communities are indicated with their average light intensity. Abbrevations are explained in Figure 2

species sorting and physiological plasticity can be partially disentangled, and (b) multiple variables such as light intensity, light variability, and nutrient supply contributed to shifts in fatty acid and species composition of the studied phytoplankton communities.

4.1 | Species sorting and physiological plasticity

In our experiment the nutrient supply influenced the communities the most and clearly separated between comparisons in homogeneous and heterogeneous nutrient supplied communities. Nutrients altered the competitive interactions causing species sorting or forced the organisms to adjust their physiology (species-specifically) to deal with altered environmental conditions (physiological plasticity). Here, by using the Bray-Curtis similarity index we could partially distinguish the influence of both mechanisms. Comparing the index values for fatty acid and species composition identified different implications of how the communities responded to environmental differences. On one hand the indices co-varied (indices did not differ) at low nutrient supply and different light conditions and therefore species sorting was solely at play. On the other hand both indices vary independently and therefore deviate from the 1:1 line at the same light conditions and different nutrient supply or at high nutrient supply and different light conditions indicating the importance of physiological plasticity alone.

Co-variation of both indices of communities with low P-supply indicates that (a) strong nutrient competition among the species was the driver for the species sorting, which (b) changed the community species composition and thus the fatty acid composition of the community. This interpretation fits nicely with the fact that nutrient preferences and competition advantages of species belonging to different taxonomic groups can be quite different (Litchman et al., 2012), and nutrient competition can lead to a selection for species that can grow in and cope with low nutrient supply (Holm & Armstrong, 1981), such as the green algae and the cyanobacterium used here. Both these species show a limited variety in their fatty acid profile, which could be one reason for the quite similar values of the fatty acid similarity index at low nutrient levels.

Under high P-supply conditions the species composition changed while the fatty acid composition was maintained, so that species either influenced each other or adjusted their physiology according to the environmental factors, or did both at once when light conditions differed. It appeared that with sufficient nutrient supply, communities cultivated with variable light showed a slight change in species composition, but the species were more capable of compensating in their fatty acid composition. On one hand, possibly, the higher nutrient supply made it possible for certain species to engage in physiological plasticity caused by the light factors, as nutrients were sufficiently available and light became the resource they were competing for. On the other hand, a higher nutrient concentration allows the phytoplankton species to utilize the light energy more efficiently (Hill et al., 2011; Urabe & Sterner, 1996). Therefore, when light (intensity or condition) differs in high nutrient supply resulting in a similar fatty acid composition but slightly dissimilar species composition

we suggest an increasing influence of physiological plasticity. Even if species proportions have shifted while the fatty acid composition remained similar, species are able to adjust their fatty acid profiles according to the environmental conditions.

In communities that were cultured in the same light but different nutrient supplies the species composition was more similar than the fatty acid composition. We found a clear shift in the primary mechanism from species sorting to physiological plasticity, when considering the environmental factor light condition (variability vs. constant) in addition to light intensity for communities cultured in different nutrient supplies. When the light fluctuated around a lower light intensity (105 μmol photons·m^{-2}·s^{-1}) there was only species sorting at play, whereas when light fluctuated around a higher light intensity (195 μmol photons·m^{-2}·s^{-1}) physiological plasticity became more important. A potential reason for this pattern could be that in the communities with a lower mean light intensity most species were potentially more light-limited than nutrient-limited.

Here we clearly show that physiological plasticity substantially contributes to community fatty acid composition. Overall, the effects of species sorting and plasticity appear to influence each other and possibly compensate for each other. Generally we can say that both mechanisms are of similar relevance and are mostly influenced by nutrient supply rather than light effects. This could be partially due to the relatively short timescales (for e.g. fluctuations) used in this experiment, as longer scale fluctuations have been shown to be decisive for physiological plasticity (Stomp et al., 2008). Physiological plasticity and species sorting might generally take effect on different time scales depending on the species growth rates and the physiological response. In our case, we assume that changes in fatty acids are slightly faster than changes in the community composition, but we suspect that this would not influence the general pattern. Additionally, the different harvesting times of high and low P-supplied cultures might have resulted in a slightly stronger nutrient effect, however, the effect can be expected to remain significant, when cultures were harvested at the same time.

4.2 | Fatty acid response

Most of the fatty acids and fatty acid groups were most strongly affected by nutrient supply (Nutrients separated communities along PC 1 and influenced all fatty acid groups and single fatty acids, except EPA, in the three-way ANOVA). Besides species shifts due to nutrient competition another reason for the clear nutrient influence could be that due to nutrient limitation only certain fatty acids were produced. Under low P-supply communities contained overall less TFA per carbon in concert with lower PUFA and specifically LNA and ALA proportions. This was associated with increased proportions of SFA and MUFA, and is often observed in phytoplankton that has been cultured under high light or low nutrient conditions. Such increased production of SFA- and MUFA-rich triacylglycerols as storage lipids is a mechanism to redistribute excess carbon, which cannot be used for growth, since other nutrients are lacking (Guschina & Harwood, 2009; Hill et al., 2011; Piepho et al., 2012). This could also explain why light had a smaller effect on the fatty acid composition of the communities (Light intensity separated the communities along PC 2 and influenced MUFA, LNA, ALA, and EPA in the three-way ANOVA). Several factor interactions found for ARA and EPA proportions also demonstrate that light effects were mainly present in communities grown at high P-supply. This contradicts single species experiments, where the production of several SFA and PUFA with light intensity were more distinct in low P treatments (Piepho et al., 2012). Generally, low irradiances favor PUFA production (Amini et al., 2012; González et al., 2019; Molina Grima et al., 1992; Solovchenko et al., 2008), which was in our case true solely for EPA. PUFA were not and LNA and ALA were even negatively affected by the lower light intensity. The communities cultivated at constant low light might have been growing better and used up more P and hence, by producing more EPA under low light showed more physiological plasticity. For example in *C. ovata* grown at low-light supply it has been suggested that a higher production of EPA originates from its role in chloroplast lipids (Klyachko-Gurvich et al., 1999; Piepho et al., 2012; Wacker et al., 2016). In contrast, communities grown at constant high light intensities could have grown better, but were restricted in growth by P. In cultures that experienced light fluctuations around different mean light intensity some species might have adjusted their photosynthetic mechanisms partially by producing certain fatty acids used for shade adaptation that requires an increase in structural lipids of photosynthetic apparatus membranes (Blanchemain & Grizeau, 1996; Mock & Kroon, 2002).

4.3 | Implications and conclusion

In this study, we used three different very important environmental factors that highly influence phytoplankton communities and their fatty acid composition. Additionally, we used well established diversity measures on the species and fatty acid composition to distinguish the relevance of species sorting and physiological plasticity. Although in other studies one mechanism was suggested to be of higher importance than the other, we propose that the relevance highly depends on the environmental factors that influence the system. We were able to show in our simplified scenario that ecological adjustments can be done without species turnover. Disentangling these patterns becomes more difficult the more complex the community becomes. Overall we can say that for fatty acid production nutrient supply plays a larger role than light intensity or condition. Nevertheless, light strongly affects the species and their competitive interactions, this way directly and indirectly shaping the community composition and its food quality for higher trophic levels.

The phytoplankton community response to environmental factors by physiological plasticity or species sorting is of high importance for the food quality of phytoplankton communities for higher trophic positions and hence the energy transfer in aquatic food webs. An increased nutrient input in a lake and thus a shift to a high nutrient

stage, leads to a physiological alteration of the fatty acid composition. Additionally, the nutrient input leads to increasing phytoplankton biomass which changes the light environment, inducing a further change of the species composition. This alteration of the species composition in a community can change the food quality substantially, as species show very distinct fatty acid profiles and together with the species adjustments in their fatty acid profiles can lead to a gradual regime shift marked by decreasing efficiency in trophic coupling between phytoplankton and zooplankton (Gladyshev et al., 2011; Hartwich et al., 2012; Marzetz et al., 2017; Selmeczy et al., 2019). Thereby, species-specific physiological responses to environmental factors and interspecific competition can additionally change the fatty acid composition of a community (Piepho et al., 2012; Wacker et al., 2015) and alter the nutritional quality of phytoplankton communities.

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VM and AW designed the experiment. VM performed the experiment. VM did the chemical/biochemical analysis. All authors analyzed the data. VM and AW wrote the manuscript. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Data Availability Statement

Data are available from the authors upon request.

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SUPPORTING INFORMATION

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