Testing for terrestrial microalgae productivity under elevated CO2 conditions and nutrient limitation

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Abstract

Background: Microalgae CO_2 fixation results in the production of biomass rich in high-valuable products, such as fatty acids and carotenoids. Enhanced productivity of valuable compounds can be achieved through the microalgae's ability to capture CO_2 efficiently from sources of high CO_2 contents but is dependent on the species. Although culture collections of microalgae provide a large variety of defined strains, inadequate understanding of which groups of microalgae and strains' origins from habitat offer high productivity under increased CO_2 concentrations hampers exploiting microalgae as a sustainable source in the bioeconomy.

Results: Growth under atmospheres of CO_2 levels of 5 - 25 % in air was examined for 12 new microalgae isolates from adverse terrestrial environments and 69 strains from the culture collection of algae of Göttingen University (SAG), preselected from about 200 strains for their phylogenetic diversity and high productivity under ambient CO_2. Green algae from terrestrial environments, including the new isolates, exhibited enhanced growth up to 25% CO_2 atmosphere. In contrast, in unicellular red algae and stramenopile algae, which originated through the endosymbiotic uptake of a red algal cell, growth at CO_2 concentrations above 5% was suppressed or ceased. While terrestrial stramenopile algae generally tolerated such CO_2 concentrations, their counterparts from marine phytoplankton did not. The tests of four new terrestrial strains in submersed culture revealed enhanced biomass and chlorophyll production under elevated CO_2 levels. The 15% CO_2 aeration increased their total carotenoid and fatty acid contents, which were further stimulated when combined with starvation in macronutrients, i.e., less with phosphate and significantly more with nitrogen-depleted culture media.

Conclusion: Isolates of green algae from terrestrial environments, Chlorophyceae and Trebouxiophyceae, exhibit enhanced productivity of carotenoids and fatty acids under elevated CO_2 concentrations. This ability supports the economic and sustainable production of valuable compounds of these microalgae using inexpensive sources of high CO_2 concentrations such as industrial exhaust fumes.

Background

Microalgae, being able to store energy from sunlight and fundamental in the global carbon cycle, have attracted worldwide attention in biotechnology. Microalgae CO_2 fixation is accompanied by the production of biomass which can be transformed into a great variety of high-valuable products, such as polyunsaturated fatty acids and carotenoids, e.g., [1-4]. Microalgae are accepted as a significant alternative source for renewable fuels [5, 6], and biogas [7]. They can also be efficiently employed in bioremediation processes, such as wastewater treatment and greenhouse gas mitigation [1]. Among the many advantages of microalgae is their high photosynthetic efficiency, resulting in fast growth and increased productivity [8]. The ability to tolerate high CO_2 contents (5% to 15% and even higher) allows microalgae to capture CO_2 efficiently from streams such as flue gases and flaring gases. Still, it is dependent on the species of microalgae [5, 9]. The concentration of CO_2 in power plant exhaust fumes

may vary between 6–13%, depending on the fuel composition [10]. In addition, or as an alternative to chemical and physical approaches to reduce the CO_2 in exhaust fumes, the growth of microalgae can considerably mitigate the CO_2 contents of exhaust fumes [11, 12]. Approaches aiming at biofuel production [13, 14] also require algae strains, which produce biomass under high carbon dioxide concentrations.

In the emerging field of microalgae-based processes and products, culture collections of microalgae are important resource centers providing a wide variety of defined algal strains. Only defined culture strains of microalgae can ensure reproducibility. Because of their purity, genetic stability, and constant availability, they meet the high-quality standards required for bioeconomy (e.g., [15, 16]). However, only culture strains that have proven their agitation resistance and high productivity in liquid culture, preferably with simple, inexpensive growth media, appear suitable for the economic growth in industrial photobioreactors. Algal strains that are productive under elevated levels of CO_2 in air are of particular interest because they offer industrial exhaust fumes to be used as an inexpensive source of CO_2 and for agitating the algal suspension in photobioreactors.

Screenings for algal strains tolerating elevated CO_2 concentrations have already been performed previously (e.g., [17, 18]). Almost all previous studies have used either small numbers of strains or a small phylogenetic diversity of algal strains. The experimental conditions used ranked from ambient air up to 20% CO_2 in air while temperature, culture media, and illumination were varied. A mixed biodiverse microalgae community has been exposed to flue gas and *Desmodesmus* spp. (Sphaeropleales, Chlorophyceae) were the main surviving species after several months [13, 19]. A screening of 12 microalgal strains was performed at 2% CO_2 in air, and green algae of the Chlorophyceae, i.e., *Chlamydomonas* spp. (Volvocales) and *Tetradesmus obliquus* (Sphaeropleales), found most suitable for biodiesel production [14]. The lipid production of the green algae *Botrycoccus braunii*, *Chlorella vulgaris* (both Trebouxiophyceae), and *Scenedesmus* spp. (Sphaeropleales, Chlorophyceae) under 10% CO_2 revealed species-specific lipid production [20].

The SAG culture collection has provided the public with pure, defined culture material for almost 70 years. Therefore, expertise has accumulated that microalgal strains isolated from various terrestrial habitats (e.g., soil and rock surfaces) are more robust than those from phytoplankton, i.e., are sufficiently agitation resistant and productive in simple liquid mineral culture media when bubbling with air at ambient CO_2. In searching for robust and productive algal strains suited for the photobioreactor technology [21] under elevated levels of CO_2 in the air, we isolated 12 new strains from algal blooms on the surfaces of poor and unfavorable terrestrial habitats. Such environments may promote the growth of particularly robust microalgal species. In addition, we performed a screening of defined and already available microalgae strains from the SAG culture collection, representing different classes of algae. We tested selected examples from the Cyanobacteria, Rhodophyta, and stramenopile algae (Eustigmatophyceae, Xanthophyceae, and the diatom *Phaeodactylum*). All tested algal strains are quickly and constantly accessible from the SAG culture collection [15] or other culture collections (Fig. 1). The algal strains were

maintained on agar plates exposed to atmospheres of 5-25 % CO_2 in air, which allowed for defined conditions independent of the CO_2 gas dissolution in the liquid phase [22]. Finally, we selected four strains from the new terrestrial isolates, which concerning CO_2 tolerance and productivity were most promising for CO_2 aeration in submerged culture as required for photobioreactor technology. We explored their production of valuable compounds, i.e., carotenoids and fatty acids, under gassing of air with elevated CO_2. Finally, the selected strains were subjected to starvation in macronutrients, i.e., phosphorous and nitrogen, to test whether this could further stimulate valuable compound productivity in combination with elevated CO_2 concentrations.

Results

Identification of the newly isolated strains

Sequence comparisons of the ITS2 rDNA revealed the 12 new green algal strains to share high similarities, i.e., 95 -100%, with available references (Table 1). This identified the strains as four different species of *Tetradesmus* (*T. arenicola*, *T. bajacalifornicus, T. deserticola*, and *T. obliquus*), two species of *Desmodesmus* (*D. armatus* and *D. multivariabilis*), *Pseudomuriella aurantica*, and *Chlorella vulgaris*. The strain SAG 2630 shared high sequence similarity (98 %) with an unidentified *Chlamydomonas* sp. (Volvocales, Chlorophyceae) and, therefore, was left unidentified at the species level.

Table 1

The 12 newly isolated strains, their species identification, origins, sequence accessions, and closest reference sequences.

Strain	species identification	isolation source; latitude, longitude	sequence accession no.	length ITS2	ITS2 sequence identities with next closest reference	sequence accession no. next closest reference
SAG 2630	*Chlamydomonas* sp.	Temporary freshwater pond with algal bloom (Israel, Haifa); 32.808119 N, 35.020541 E	MZ546610	234	230/235 (98%)	MH311547
SAG 2629	*Chlorella vulgaris*	Temporary freshwater rivulet, iron-rich (Germany, Bad Pyrmont); 51.988757 N, 9.252696 E	MZ546604	244	244/244 (100%)	AY591499, and 55 other
SAG 2606	*Chlorella vulgaris*	Temporary freshwater rivulet, iron-rich (Germany, Bad Pyrmont); 51.988757 N, 9.252696 E	MZ546608	243	244/244 (100%)	AY591499, and 55 other
SAG 2635	*Desmodesmus armatus*	Soil surface of a meadow (Ukraine); 46.480278 N, 33.849722 E	MZ546611	244	245/245 (100%)	MK975484, and 12 other
SAG 2628	*Desmodesmus multivariabilis*	Biofilm on soil surface (Germany, Uslar); 51.649100 N, 9.750686 E	MZ546603		248/248 (100%)	MH311545
SAG 2631	*Pseudomuriella aurantiaca*	Biofilm on soil surface (Israel, Haifa); 32.778451 N, 35.025604 E	MZ546609	246	245/247 (99%)	MH703741, and 2 other
SAG 2632	*Tetradesmus arenicola*	Biological soil crust on the surface of sandy soil (Ukraine); 46.576798 N, 31.512473 E	MZ546602	239	240/240 (100%)	MH703775, and 3 other
SAG 2633	*Tetradesmus arenicola*	Surface of sandy soil (Ukraine)	MZ546612	239	240/240 (100%)	MH703775, and 3 other
BIOTA 136	*Tetradesmus bajacalifornicus*	Biological soil crust on the surface of sandy soil (South Africa, semi-	MZ546605	242	231/244 (95%)	HQ246450, and 4 other

		desert); 30.1865 S, 17.5433 E				
BIOTA 153	*Tetradesmus deserticola*	Biological soil crust on the surface of sandy soil (South Africa, semi-desert); -30.3856 N 18.2757 E	ON677848	240	234/240 (98%)	AY510471
SAG 2608	*Tetradesmus obliquus*	Biofilm on soil surface next to a freshwater pond (Germany, Uslar); 51.647250 N, 9.761198 E	MZ546606	239	240/240 (100%)	MK975482, and 52 other
SAG 2607	*Tetradesmus obliquus*	Biofilm on soil surface next to a freshwater pond (Germany, Uslar); 51.647250 N, 9.761198 E	MZ546607	239	240/240 (100%)	MK975482, and 52 other

Growth under elevated CO_2 atmospheres

All the 12 new green algal strains from terrestrial habitats exhibited robust growth under the atmospheres of elevated CO_2. *T. bajacalifornicus* BIOTA 136 showed even enhanced growth at all tested levels of elevated CO_2 atmospheres (growth pattern 7, Fig. 1). *D. armatus* SAG 2635 and *D. multivariabilis* SAG 2628 were tolerant to the 5 and 15 % CO_2 atmospheres and exhibited enhanced growth under the 25 % CO_2 atmosphere (growth pattern 6, Fig. 1). The new strains of *T. obliquus*, SAG 2607 and SAG 2608, had enhanced growth under 5 % CO_2 but tolerated the higher CO_2 levels (growth pattern 6, Fig. 1). In contrast, the growth of other species of *Tetradesmus*, i.e., *T. arenicola* and *T. deserticola*, was suppressed under 5 % and 15 % CO_2 atmospheres, although it did not cease under 25 % CO_2. Growth of the new *C. vulgaris* strains SAG 2606 and SAG 2629 showed nor or just few adverse effects under the tested elevated CO_2 atmospheres (growth pattern 5, Fig. 1). Similar growth patterns, i.e., suppressed growth under the 15 % CO_2 atmosphere, were found for the new strains of *Chlamydomonas* sp. SAG 2630 and *P. aurantiaca* SAG 2631 (growth patterns 4 and 2, Fig. 1).

Most other green algal strains already available from culture collections were also robust towards atmospheres of elevated CO_2 conditions. Even at 25 % CO_2, increased growth was observed for *Desmodesmus komarekii* strain CCAP 258/232 (growth pattern 6, Fig. 1). Most frequent were the growth patterns with no adverse effects at all three elevated CO_2 levels, found in strains from all three tested classes of the Chlorophyta (growth patterns 5 and 4; Fig. 1). Among the already available green algal strains from the SAG culture collection, a number of those from the Chlorophyceae and Trebouxiophyceae tolerated just the 5% CO_2 atmosphere while reacting with suppressed growth under higher CO_2 levels, e.g., the tested strains of *Ettlia carotinosa* and *Neocystis brevis* (growth pattern 3, Fig.

1). Out of the Chlorophyceae, only *Chlorococcum novae-angeliae* strain SAG 5.85 reacted with suppressed growth under all atmospheres of elevated CO_2 (growth patterns 2, Fig. 1). Among the tested strains of Trebouxiophyceae, only the growth of *Chloroidium angusto-ellipsoideum, Coccomyxa avernensis, Lobosphaera incisa, Stichococcus ampulliformis,* and two strains of *S. bacillaris*, was suppressed under elevated CO_2 atmospheres (growth pattern 2, Fig. 1).

For all tested Cyanobacteria strains, CO_2 atmospheres higher than 5% resulted in suppressed growth (growth patterns 3 and 2, Fig. 1). Two cyanobacteria strains did not grow, even under the 5 % CO_2 atmosphere (growth pattern 1, Fig. 1). The tested strains representing unicellular red algae (Rhodophyta) had growth patterns under elevated CO_2 atmospheres like those of the Cyanobacteria. In about half of the tested red algal strains, 5% was the only level of elevated CO_2 tolerated, while all higher CO_2 levels led to suppressed growth (growth patterns 3 and 2, Fig. 1). However, just for one single strain, *Porphyridium purpureum* SAG 1380-1a, the 25 % CO_2 atmosphere did not affect its growth.

Among strains of stramenopile algae, class Eustigmatophyceae, the strains from terrestrial habitats of the genus *Vischeria* tolerated 5 % CO_2, with *V. polyphem* strain SAG 38.84 even 15 % CO_2 in the atmosphere (growth patterns 3 and 5, Fig. 1). However, the growth of the tested strains from marine phytoplankton, the genera *Microchloropsis* and *Nannochloropis*, was suppressed, or it even ceased at all elevated CO_2 levels (growth patterns 2 and 1, Fig. 1). It confirms earlier studies on the CO_2 utilization of *N. oculata* in response to CO_2 aeration [23]. From the tested stramenopile algal strains of the class Xanthophyceae, only a single strain of a typical terrestrial (soil) alga, *Heterococcus viridis* SAG 2422, was left unaffected in growth until 15% CO_2 in the atmosphere. However, *Ophiocytium parvulum* strain SAG 37.84 from an aquatic environment tolerated only the 5% CO_2 atmosphere. The other tested Xanthophyceae strain reacted with suppressed or even ceased growth to elevated CO_2 levels in the atmosphere (growth patterns 5, 3, 2, and 1, Fig. 1). Among the three tested strains of the diatom *Phaeodactylum tricornutum*, a diatom isolated from marine or brackish environments, the growth of two strains was suppressed under all tested CO_2 levels (growth patterns 4 and 2, Fig. 1). Only one strain, SAG 1090-6, did not exhibit adverse effects on the growth under the 5 and 25 % atmospheres.

Productivity of selected green algal isolates of terrestrial habitats in submersed culture

We selected four of the new green algal isolates as examples to further test terrestrial microalgae (including one from a temporary freshwater rivulet) for their productivity of valuable compounds. To resemble processes in photobioreactors, we performed the tests in submersed culture, i.e., liquid culture medium that was aerated with CO_2 at either ambient or 15% concentration. One selected strain was *Tetradesmus bajacalifornicus* SAG BIOTA 136, the only strain exhibiting enhanced growth under all elevated CO_2 atmospheres (growth pattern 7, Fig. 1). Two more strains were *T. obliquus* SAG 2607 and SAG 2608, which differed in their tolerance towards 15 % CO_2, i.e., with growth unaffected (SAG 2607) or slightly enhanced (SAG 2608) (growth pattern 6, Fig. 1). Finally, *Chlorella vulgaris* strain SAG 2606 represented those strains tolerating all three tested CO_2 levels without change in growth (growth pattern 5,

Fig. 1), the most common growth pattern among the tested green algal strains. We analyzed the effects along with CO_2 levels from ambient to 15% in the aeration on the productivity of biomass and chlorophyll (Fig. 2), followed by the impact of aeration of 15% CO_2 compared to that with ambient CO_2 on the contents of carotenoid (Fig. 3) and total fatty acid contents (Fig. 4, top). Finally, we analyzed the same CO_2 effects on the ten selected single fatty acids, which included 8 polyunsaturated fatty acids

(PUFAs; Fig. 4), measured as fatty acid methyl esters (FAMEs). A prerequisite for the tests in submerged culture was to ensure that the found effects would concern just those caused by the enhanced (15%) CO_2 concentration. There is the possibility that changes in the pH value generated by the CO_2 aeration may interfere as a selection criterion. However, only a relatively small alkalization was observed due to the CO_2 aeration from ambient to 15% CO_2 (Additional file 2: Fig. S2). The consumption of CO_2 and nitrate largely overcompensated the potential acidification. The metabolism of both leads to alkalization of the medium, based on an H^+- cotransport (in the case of nitrate) or Na^+- cotransport (partly for CO_2) via the plasma membrane. Therefore, the pH remains in the buffer area of the carbonate buffer system (pH 6.5) [22]. This also applies to cultivation on agar plates, the culture medium (20 mL) of which has the same buffer capacity as the liquid culture.

Increasing the CO_2 supply stimulated biomass production in a liquid medium dependent on the CO_2 concentration in all four strains (Fig. 2). A continuous increase in biomass with increased CO_2 supply was observed for *C. vulgaris* and the two *T. obliquus* strains. In contrast, *T. bajacalifornicus* achieved its highest biomass productivity at 2% CO_2 and there was no further increase with higher CO_2 concentrations (Fig. 2). The chlorophyll content in the three *Tetradesmus* strains (Fig. 2) went up sharply at 1% CO_2 to a level where almost no alterations occurred with further increasing CO_2 concentrations. However, in *T. obliquus* SAG 2608, chlorophyll content decreased at 10 and 15% CO_2, almost to the level under ambient CO_2 concentration (Fig. 2). In *C. vulgaris* SAG 2606, there was a similar sharp increase to a level at which further supply of CO_2 had hardly any effect.

Carotenoid contents under ambient CO_2 (condition AC) were almost double as high or higher in the three *Tetradesmus* strains than in *C. vulgaris* (Fig. 3). Aeration with 15% CO_2 (condition CC) nearly doubled the total carotenoid content in all four strains compared to ambient CO_2 in air (condition AC). Carotenoid production may be a significant sink for excess carbon under elevated CO_2 supply in these strains. Concerning total carotenoid content, *C. vulgaris* was less productive than the other three strains (Fig. 3).

Total fatty acid content, i.e., the sum of the ten measured fatty acids (Additional file 3: Table S1) under ambient CO_2 (condition AC), was highest in *T. bajacalifornicus* compared to the other three tested strains (Fig. 4, top). Total fatty acid production increased with 15% CO_2 (condition CC) in all four strains. The effect was most pronounced in *C. vulgaris,* where the entire fatty acid content almost doubled, whereas there was only a slight increase (about 15-30%) in the three *Tetradesmus* strains (Fig. 4, top).

We further investigated the content of 10 fatty acids, measured as FAMEs, in all four strains under ambient and elevated CO_2 (conditions AC and CC; Fig. 4, Additional file 3: Table S1). *C. vulgaris* exhibited the highest 18:3α content. Also, *C. vulgaris* was most rich in the 16:3 fatty acid, followed by *T. bajacalifornicus* under both conditions, AC and CC. *C. vulgaris* differed from the other three strains that no 18:4 was found (Fig. 4). In all strains, an increase in 18:1 9z due to 15% CO_2 aeration was observed. In general, the effect of elevated CO_2 aeration on the measured fatty acids was most pronounced in *C. vulgaris* SAG 2606. There, the elevated CO_2 gassing resulted in an increased content of all except the 16:4 measured fatty acids (Fig. 4). In contrast, in the three *Tetradesmus* strains, an increment due to elevated CO_2 was found in only some fatty acids. In the three *Tetradesmus* strains there was little or no elevated CO_2 (condition CC) effect for 16:0 and 18:2 LA with elevated CO_2 (condition CC). In *T. bajacalifornicus* BIOTA 136 the contents of 16:3. 16:4, 18:3α, and 18:4 fatty acids were increased while in the *T. obliquus* strains only those of the 18:0, and 18:1(n-9) (oleic acid) fatty acids were increased (Fig. 4). The positive effect for 18:4 clearly seen in *T. bajacalifornicus* was rather little in the two *T. obliquus* strains (Fig. 4).

Effects of macronutrient limitation on the productivity of the four selected terrestrial strains

Macroelement limitations for nitrogen and phosphorus were applied separately to test whether these manipulations could further increase or decrease the effects of elevated CO_2 supply on the content of carotenoids and fatty acids. Under nitrogen limitation and ambient CO_2 (condition A-N), the carotenoid content increased in all four strains compared to that obtained in the complete medium (condition AC) (Fig. 3). Aeration with 15% CO_2 under nitrogen limitation (condition C-N) increased the carotenoid content in all four strains. However, phosphate starvation under ambient CO2 (condition A-P) did not affect total carotenoid contents in all four strains compared to complete medium (condition AC), i.e., they remained at about the same level. Elevated CO_2, in addition to phosphate starvation (condition C-P), doubled the total carotenoid content compared to ambient CO_2 in *C. vulgaris* and *T. obliquus*. However, it only slightly increased in *T. bajacalifornicus* (Fig. 3). Generally, the values obtained from phosphate starvation combined with elevated CO_2 (condition C-P) were lower than those obtained under nitrogen limitation (condition C-N; Fig. 3).

Total fatty acid content was strongly stimulated in all four strains by N-limitation alone (condition A-N; Fig. 3) compared to their growth in complete medium (condition AC). However, the combined treatment of elevated CO_2 and N-limitation (condition C-N) further increased total fatty acid content only in *C. vulgaris* (Fig. 3; Additional file 3: Table S1). From the detailed analysis, it was apparent that N-limitation alone, condition A-N, had a robust, increasing effect on 18:1 9z compared to the complete medium (condition AC) in all four strains (Fig. 4; Additional file 3: Table S1). However, the combined treatment, condition C-N, reduced the amount of 18:1(n-9) in the three *Tetradesmus* strains compared to N-limitation alone (condition A-N), but not in *C. vulgaris* where the combination still led to increased 18:1 9z content. Also, the amount of 18:0 decreased under the condition C-N in all four strains compared to that at ambient CO_2 (condition A-N) (Fig. 4; Additional file 3: Table S1). There was an increase in the contents of 16:3 and

18:3α in all strains under the combined treatment (condition C-N). It was pronounced in *C. vulgaris*. The content of 18:4, the fatty acid was not found in *C. vulgaris*, and it was consistently higher under the combined treatment (condition C-N) in the two *T. obliquus* strains. The 18:4 fatty acid content was significantly increased in *T. bajacalifornicus* under the combined treatment (condition C-N). While N-limitation alone (condition A-N) raised the 18:0 fatty acid concentration considerably, the additional supply of CO_2 (condition C-N) reduced its content (Fig. 4; Additional file 3: Table S1). The content of the 16:4 fatty acid was low in *C. vulgaris* under condition AC and not detectable under N-limitation, condition A-N (Fig. 4; Additional file 3: Table S1). In contrast, the *Tetradesmus* strains exhibited higher contents of the 16:4 fatty acids under the AC condition. Among those, nitrogen limitation reduced the 16:4 fatty acid content only in *T. bajacalifornicus* (Fig. 4; Additional file 3: Table S1).

P-limitation under ambient CO_2 (condition A-P) only slightly increased total fatty acids compared to the complete medium, i.e., condition AC. (Figs 3, 4; Additional file 3: Table S1). In combination with elevated CO_2 (condition C-P), fatty acid production was further expanded in *C. vulgaris* and *T. obliquus* but reduced in *T. bajacalifornicus* (Fig. 4). The absolute contents of all fatty acids under P-limitation corresponded quite well to their respective contents with complete media (Fig. 4). The detailed analysis showed a slight increase of content in all fatty acids (conditions A-P, C-P), except 18:1(n-9) and 18:0 in the *T. obliquus* and *C. vulgaris* strains (Fig. 4). At the same time, it decreased in *T. bajacalifornicus* (Fig. 4; Additional file 3: Table S1). We conclude that P-limitation had only negligible effects on the fatty acid contents. In all strains, the N-limitation (condition A-N) had the most significant impact on the contents of all ten tested fatty acids (Fig. 4). Especially, the 18:1 9z was increased under ambient CO_2 (condition A-N) (Fig. 4; Additional file 3: Table S1).

Discussion

Growth experiments under elevated CO_2 atmospheres

Our study aimed at identifying algal strains that can sustain or even exhibit positive responses to higher CO_2 concentrations. Such strains will be useful for economical production of high contents of valuable substances. Industrial exhaust fumes, which are about 15% CO_2 [24], could be used with theses strains as a CO_2 source. Our study's newly isolated green algal strains tolerated atmospheres of elevated CO_2 concentrations. Although some strains reacted with somehow suppressed growth, they did not cease it completely (growth patterns 2 and 4; Fig. 1). Most strains showed no effects (growth pattern 5) or even enhanced growth (patterns 6 and 7) compared to ambient CO_2 concentration. The new green algal isolate, *Tetradesmus bajacalifornicus* strain BIOTA 136, from a Biological Soil Crust in an arid area of South Africa exhibited enhanced growth under all elevated levels (growth pattern 7, Fig. 1). It even outperformed other strains of the same genus and those of the closely related *Desmodesmus* spp. (Fig. 1). It suggests that green microalgae isolated from unfavorable terrestrial habitats, such as blooms on soil surfaces or biological crusts of arid environments, provide robust strains productive under elevated levels of CO_2. Those strains may be well suited for using flue and flaring gases as cheap CO_2 sources

and agitation of the cell suspensions using photobioreactor technologies. The tested green algal strains isolated from terrestrial habitats can tolerate CO_2 levels beyond the relatively low threshold of 2-5 % considered as saturating for the CO_2 uptake of the overwhelming majority of photoautotrophs [18]. Previous studies found only relatively few strains able to sustain growth under higher CO_2 concentrations. Among those were several strains of Sphaeropleales (Chlorophyceae), i.e., *Desmodesmus* and *Tetradesmus* ("*Scenedesmus*") [9]. Our study expands the recovered phylogenetic breath of *Tetradesmus* species well suited for CO_2 mitigation [9] by the addition of new strains of *T. bajacalifornicus* and *T. obliquus*. Furthermore, the new isolate from *Chlorella vulgaris* SAG 2606 was even more productive with respect to fatty acids under elevated CO_2 than the tested species of *Tetradesmus* (Fig. 4). In other previous studies, only 2 out of 74 (2.7 %) and 17 out of 96 (17.7 %) test strains were found tolerant [25, 26]. Among the test strains of our study, however, a total of 34 strains (39.5 %) reacted positively concerning CO_2 tolerance or even improved growth.

In contrast to green algae (Chlorophyceae and Trebouxiophyceae), unicellular red algae were found sensitive to CO_2 levels higher than 5% in air. The tested stramenopile algae (Xanthophyceae, Eustigmatophyceae, and *Phaeodactylum*) exhibited similar behavior. The latter originated from secondary endosymbiosis with a red alga. The three Eustigmatophyceae strains from the marine environment, *Microchloropsis,* and *Nannochloropsis*, which ceased growth or showed suppressed growth under elevated CO_2 levels were the only test strains originating from marine phytoplankton. Marine phytoplankton algae may generally not be suited for growth under exhaust fume gassing, but this point needs to be tested with a broader range of culture strains. In contrast, the terrestrial representatives of stramenopile algae tested here (*Vischeria*, *Heterococcus*) were mostly found adapt better with enhanced growth or at least exhibited tolerance at 5% CO_2 in air (Fig. 1). Cyanobacteria strains generally showed adverse effects towards elevated CO_2 (Fig. 1). Also previous growth experiments with the cyanobacteria *Microcystis aeruginosa* and *Anabaena spiroides,* showed inhibitory effects under elevated CO_2 [27]. The reason for this inhibition remains unclear.

Among the tested algal strains, the green algae (Chlorophyta), which comprised the most extensive group of strains, was the algal group with the smallest proportion of strains that exhibited adverse effects (41.2 % or 21 strains; Fig. 1) under the elevated CO_2 atmospheres. Various green algal strains from the order Sphaeropleales (Chlorophyceae), i.e., all 12 tested strains of *Tetradesmus* spp. and *Desmodesmus* spp., were confirmed as the most promising green algal strains. In our growth experiments, they were the only strains that exhibited the two best growth patterns (6 and 7; Fig. 1), featuring tolerance towards 15% and 25 % CO_2 coupled with growth enhancement under at least one other elevated CO_2 level (Fig. 2). Similar findings have been reported from previous studies [9, 28-30]. Members of Sphaeropleales, as well as *Chlorella vulgaris* (Trebouxiophyceae), have been considered as promising sources of biodiesel [9, 13, 14, 31, 32]. For the new strain BIOTA 136 there were five closest reference strains representing *T. bajacalifornicus* [33] and the maximum ITS2 sequence identity with them was 95 %. This is below the range of sequence identities among the so far available strains of that species, which is 97 - 100%.

Therefore, additional sequence analyses, e.g., with the UPA and *rbc*L genes [33], are required to further investigate the species identity of strain BIOTA 136.

We employed algal growth on agar plates to expose 81 test strains to atmospheres of various elevated CO_2 concentrations in air. Plastic bags filled with the atmospheres of different CO_2 concentrations have been used to grow the algae in the liquid medium [26]. Although both methods appear suitable for an extended screening, only the simple approach using agar plates allows for the extended duration of the cultivation as the nutrient supply may not become limiting within a short time [34]. The visual assessment of algal growth allowed us to assign the grades to the observed productivity. Comparisons with the control under ambient CO_2 allowed for a robust estimation of the growth effects. The procedure was repeatedly tested by several independent investigators and resulted in convergent, stable assessments of the growth effects.

Production of biomass, carotenoids, and lipids in 4 selected strains

We identified four green algal strains with enhanced growth under elevated CO_2 in submersed culture. The use of 15% CO_2 in our experiments fits well with the range of the CO_2 concentrations present in industrial flue gas [35, 36], which can serve as a cheap source of CO_2. All four selected strains produced increased amounts of biomass at 15% CO_2. However, biomass alone is often not a sufficiently high value, particularly in competition with agriculture, where biomass production is more economical. Therefore, it was of interest to find compounds of added higher values. Of particular interest are products useful for human nutrient supply and raw material for pharmaceutics for which lipids and pigments are considered [37]. Algae as cell factories produce both types of compounds, but their content may frequently not be sufficiently high [38]. The supply of enhanced CO_2 concentrations from cheap sources may increase the production of total lipids and total carotenoids due to photosynthesis stimulation [39]. Then algal biomass may also serve the production of biofuels [40, 41], and other high-value compounds [42, 43]. Due to the high CO_2 supply, the most significant increase in total lipids have been observed with the strain *Chlorella vulgaris* SAG 2606, while the three *Tetradesmus* strains exhibited higher carotenoid contents (Fig. 3).

To further enhance carotenoids and lipid contents, we manipulated the supply of macronutrients, i.e., nitrogen or phosphate according to [44, 45]. Nutrient deficiency, salt stress, and deficiency in trace elements are considered as triggers for lipid accumulation [46, 47]. In the absence of nitrogen and with sufficient light, the cells continue to fix CO_2 but cannot synthesize proteins, which explains the accumulation of nitrogen-poor but carbon-rich storage substances such as lipids and starch [39, 48, 49]. The high consumption of NADPH for fatty acid synthesis additionally prevents an over-reduction of the photosynthetic electron transport [50]. In addition, photo-oxidative stress is prevented, which can damage photosynthesis.

In all four selected test strains, reduction of the nitrogen concentration (conditions A-N and C-N) resulted in a significant increase in the total lipid content compared to that in complete nutrient solution under

ambient CO_2 (Fig. 3). However, a further increase in total lipid content due to the combination of N-deficiency with 15% CO_2 in air was only observed in *C. vulgaris* SAG 2606. Such modifications in the fatty acid profile between different phyla, classes and genera could be anticipated and have been described even for species, although that was mainly based on the different cultivation conditions [51]. That is similar to the data reported by Li et al. (2012) for *Chlamydomonas,* where N-deprivation caused an accumulation of triacylglycerol (TAG). CO_2 supply up to 25% promoted cells of *T. bajacalifornicus* strain BBKLP-07 to produce high lipid contents [52]. These authors have not considered whether the stimulation of the lipid production can be further increased by nutrient starvation. In the three *Tetradesmus* strains tested in our study N-depletion under elevated CO_2 (condition C-N) caused a higher carotenoid synthesis (Fig. 3). It looks like the *Tetradesmus* strains have a different strategy for dealing with oxidative stress than *Chlorella* in that the carotenoids detoxify the oxidative singlet oxygen [42]. The oxidative stress increases as photosynthesis is stimulated under 15% CO_2, and additional N-deficiency supports the risk of photo-oxidation [50].

The supply of increased CO_2 to the selected green alga strains strongly influenced their production of omega fatty acids. When combined with nitrogen starvation, the total content of omega fatty acids increased compared to that in complete culture media under elevated CO_2 conditions. The strain *C. vulgaris* SAG 2606 displayed the substantial effect in that respect (Fig. 3). It is obviously possible to stimulate the cells to convert the supply of high levels of CO_2 into unsaturated fatty acids, especially under nitrogen limitations. A similar observation was reported by [53]. Fatty acid production increased beyond the well-known effect of nitrogen deficiency. The detailed analysis of fatty acids showed a relative increase in 16:0, 16:3, and 18:3α polyunsaturated fatty acids (PUFAs under nitrogen deficiency (Fig. 4; Additional file 3: Table S1). The ratio between the 16:3 and 18:3α fatty acids was considered to be important for the configuration and fluidity of the thylakoid membranes [54]. A high proportion of unsaturated fatty acids contributes to maintaining the fluidity, especially of the thylakoid membrane, since it has a very high proportion of membrane-integrated and -associated proteins [55]. The increased 16:0 content accumulates in monogalactosyl diacylglycerol (MGDG), which indicates a rearrangement of the chloroplast membrane (for review, [18]). We also found an 18:1 9z increase in *C. vulgaris* (Figure 4; Additional file 3: Table S1). It has also been reported for *Chlamydomonas,* where the authors correlate this result to the fast growth of algal cultures [56]. N-deprivation in combination with increased CO_2, lead to considerably enhanced content of 18:1 9z content was strongly enhanced. The omega 9 fatty acid 18:1 9z is the most common fatty acid as a component of TAGs in structural lipids.

We also reduced the phosphate supply, which in general resulted in a phenomenon similar to that as nitrogen depletion. Still, the effects were much smaller than under nitrogen limitation. The network of regulation concerning nitrogen and phosphate metabolism affecting each other, especially in case of limitation, likely is the reason [57]. The cytosolic Ca^{2+} pool is affected and dampened by phosphate starvation but not by nitrogen starvation, as reported for *Arabidopis* roots [58]. For the diatom *P. tricornutum*, phospholipids and polyphosphates can serve as phosphate storage pools. These can be

used during phosphate deficiency and therefore delay the symptoms of phosphate starvation [49] compared to the fast occurrence of N-depletion symptoms [59].

Conclusions

Growth patterns of a wide variety of defined microalgal strains from the SAG culture collection show tolerance and even growth enhancement when exposed to atmospheres of elevated CO_2 concentrations. In particular, the isolates of green algae, Chlorophyceae and Trebouxiophyceae, from terrestrial habitats such as soil surfaces or temporary freshwater bodies exhibit enhanced productivity of carotenoids and fatty acids (including PUFAs) under elevated CO_2 concentrations. This contrasts with cyanobacteria, unicellular red, and most stramenopile algae, whose growth is suppressed by elevated CO_2 levels. In green algae of the Sphaeropleales (Chlorophyceae), i.e., *Tetradesmus* and *Desmodesmus*, and *Chlorella vulgaris* (Trebouxiophyceae), aeration with elevated CO_2 into submerged culture not only increases their productivity in terms of biomass, but also in the contents of carotenoids and total fatty acids, including omega-3 fatty acids. The contents of those valuable compounds can even be increased by macronutrient starvation, especially nitrogen. These findings recommend certain green algae originating from adverse terrestrial habitats for the economic and sustainable production of valuable compounds using inexpensive sources with high CO_2 contents, such as flue and flaring gases.

Methods

New isolates, identification by sequence analyses, and tested strains

To test whether terrestrial microalgae from poor and unfavourable terrestrial habitats may provide productive and CO_2 tolerant strains, 12 new strains of green algae were established (Table 1). They were collected from algal blooms on soil surfaces including those in arid environments or temporary shallow freshwater bodies of urban environments impaired by moderate pollution. Two novel strains, BIOTA 136 and BIOTA 153, were isolated from biological soil crusts of arid climate regions in South Africa [60], and kindly provided by BIOTA, a long-term joint research project on biodiversity assessment (www.biota-africa.org). They are maintained at the SAG culture collection (Göttingen, Germany). For isolating the algal strains, standard procedures as previously described [61, 62], were applied. For identification, the ITS2 rDNA regions of the new isolates were sequenced. Amplicons spanning from the 3'-end of 18S rRNA over the ITS1, 5.8S rRNA, and ITS2 regions to the 5'-end of the 26S rRNA gene, were generated and sequenced as previously described [62, 63]. The ITS2 regions, about 238 - 244 base pairs long, were extracted from the obtained sequences using the *ITSx* software [64] in combination with own scripts. For species identification, the ITS2 sequences were queried on the portal of the NCBI Genbank database (https://www.ncbi.nlm.nih.gov/genbank/) using BLASTN [65]. The newly determined sequences are available from Genbank (Table 1).

About 200 strains, already previously available from the SAG culture collection, including one strain from the Culture Collection of Algae and Protozoa (CCAP; www.ccap.ac.uk), for which productive growth on

simple mineral media has been recorded over the many years of their maintenance, were re-examined for their growth properties before our study. Out of those, a selection of 69 strains appeared promising for testing their growth under atmospheres of elevated CO_2 levels, which our study aimed at (Fig. 1). Finally, a total of 81 strains were tested for their growth properties under atmospheres of elevated CO_2 levels in air (Fig. 1; Additional file 1: Fig. S1). They were presented by green algae (Chlorophyta), representing the classes Chlorophyceae (29 strains), Trebouxiophyceae (17 strains), and Chlorodendrophyceae (1 strain), unicellular red algae (Rhodophyta; 13 strains), stramenopile algae, representing the classes Eustigmatophyceae (7 strains), Xanthophyceae (5 strains), the diatom *Phaeodactylum tricornutum* (3 strains), and Cyanobacteria (6 strains).

Growth experiments under elevated CO_2 atmospheres with 81 algal strains

We compared the algal growth of the 81 strains while maintained on agarized (1.5 %) culture media in Petri dishes (diameter about 6 cm) under the atmosphere of CO_2 of various concentrations in air, i.e., enriched by CO_2 gas (food grade; Linde, Germany) to 5 %, 15 %, and 25 % CO_2 to their controls under ambient CO_2 concentration. The algal colonies, grown on the agar surface, were exposed to the CO_2-enriched atmospheres with the petri dish lid closed, but not sealed with parafilm. This provided maximum exposure under defined growth conditions independent of the CO_2 gas dissolution in the liquid phase. With agar plates, the duration of cultivation could be extended while the nutrient supply may not become limiting. Diffusion of CO_2 into the agar could influence the pH of the growth media. However, the solution of the growth media was with a very high buffer capacity, which kept the pH sufficiently stable. The very high buffer capacity also applies to the liquid cultures, later used for four selected test strains (Additional file 2: Fig. S2).

For the growth on agar plates, the same culture media were used as for the perpetual maintenance of the stock cultures were used, i.e., 3NBBM, BG11, ASM 15, and ASM 30 (https://www.epsag.uni-goettingen.de; [66]). Algal colonies from the stock cultures mostly maintained on agarized media in test tubes at the SAG culture collection were evenly distributed on agar plates using a Drigalski spatula. Three plates of each experimental condition were used, i.e., at elevated CO_2 atmosphere, and the control at ambient CO_2 in air, were used (Fig. 1; Additional file 1: Fig. S1). Light- and temperature-controlled culture cabinets served for the growth tests. Experimental conditions and control were conducted in parallel and simultaneously in separated growth cabinets. The agar plates were randomly placed in the cabinets and their position was changed during the culturing several times to avoid biases due to a certain position in the cabinets. These were commercially available refrigerators with a glass front door (Liebherr FKvsl 3613, Germany) inside which white LED fluorescent bulbs (Osram L 8W/640 cool white) and pipes for atmosphere gassing were mounted. Light intensity was 40 µE s^{-1} cm^{-2}, with a 14:10 day/night cycle. The temperature was 21°C. A microcomputer-controlled gassing system (QCAL Messtechnik GmbH, Germany) kept the CO_2 level constant and monitored it during the growth experiments. A ventilator and arbitrary variation of the plates' positions in the chamber circumvented possible imbalances of the CO_2

concentration inside the growth chamber during an experiment. The experiments lasted about two weeks, i.e., 12 - 18 days.

At the end of a growth experiment, the agar plates' algal growth under experimental conditions and control were photographed using digital cameras (Additional file 1: Fig. S1). The visual assessment of growth was advantageous because there were no means of quantitatively removing the colonies from the agar surfaces, which would have been a prerequisite for density measurement of algal colonies suspended in liquids. Also, the clumping of the algae in liquids was unavoidable. For the visual assessment of algal development, five grades of growth were assigned. A "0" was given to no visible growth at all and bleached colonies, grade "0.5" for stagnant growth with pale colonies, and grade "1" to colonies with growth. Still, without spreading over the agar surface, grade "2" was given to colonies of bright color scattered over the agar surface, and grade "3" to those of intense dark color and densely spread over the agar surface (Fig. 1; Additional file 1: Fig. S1). The ratio of the growth grade after the experiment to that of the control was formed, and their mean values from repeated experiments were graphically displayed (Fig. 1). For example, a ratio of 1/2 meant an adverse effect of elevated CO_2 with stagnant growth, a ratio of 2/2 tolerance with no growth change under elevated CO_2 with proper growth, and a ratio of 3/1 accelerated growth due to elevated CO_2 (Fig. 1; Additional file 1: Fig. S1). Seven growth patterns were distinguished based on their growth grade ratios under 5, 15, and 25% CO_2 in air atmospheres (Fig. 1).

Growth experiments under direct gas bubbling with four selected algal strains

Four strains from the new isolates, which exhibited enhanced or unaffected growth and at the same time appeared rather productive under the 15 and 25% CO_2 atmospheres, were selected for experiments under conditions of closed photobioreactors to mimic those used for biotechnological applications. Here direct gas bubbling into the solution was used to increase the CO_2 levels of liquid culture media. Initial tests confirmed that pH does not interfere as a selection criterion in liquid culture due to the high buffer capacity of the media (Additional file 2: Fig. S2). The focus of the experiments was on 15 % CO_2 in air because this concentration is close to industrial exhaust fumes, which often reach 10-15 % CO_2 [35, 36]. Glass column photobioreactors (Kniese, Hilke Feinmechanik GmbH, Germany) of 4 cm in diameter and a volume of 400 ml to which a glass tube for gassing was attached were used [67]. The Kuhl and Lorenzen liquid culture medium ("Kuhl medium") [67] was used because of its high buffering capacity. Light from white LEDs dedicated to plant growth (sTube, Snaggi Lighting s.r.o., Czech Republic) at an intensity of 100 µE s^{-1} cm^{-2} and under a light/dark cycle of 14:10 h was applied. The glass columns were submerged in water baths kept at 25 °C.

The photobioreactors were gassed with various gas mixtures of CO_2 in air, 0.1 L min^{-1}/tube (QCAL Messtechnik GmbH, Germany), i.e., from ambient (0.04 %) to 25 %. CO_2. We were tracking the pH by supplying 10 mM buffer systems (phosphate buffer or MOPS-buffer) and daily pH control measurements. Only a slight alkalization at the end of the experiments was found (Additional file 2: Fig.

S2). The changes in pH were small, and the available forms of carbon supply were HCO_3^- and CO_3^{2-} according to the pKs of 6.5 of the carbonate buffer system [22].

In addition to variation in CO_2 supply, experiments under various nutrient supplies and combinations of both were performed. The nitrate concentration in the medium was reduced to only 5% of that of the Kuhl medium (condition "-N"). The reduced N-supply ensured that the cultures still grew well at the beginning of the experiment before they went into the N-deficiency. For experiments with phosphate deficiency (condition "-P"), the Kuhl medium phosphate buffer was replaced by MOPS buffer with the same ionic strength and buffering capacity, following [68]. The various growth conditions were labeled as the following. "AC" and "CC" indicated experiments with ambient and 15% CO2 gassing into complete liquid Kuhl medium. The labels "A-N", "C-N", and "C-P", "C-P" indicated the corresponding experiments with media reduced in nitrate and phosphate, respectively (Figs 3, 4; Fig. 1; Additional file 3: Fig. S3).

Biomass, pigment production, and lipid levels of the four selected strains.

After one week, the growth of the four selected algae strains cultivated in liquid media was determined via biomass production, measured as dry weight per mL suspension. The pigments, chlorophylls *a* and *b*, and total carotenoids, were measured. For the extraction of pigments, 2 mL of algal suspension were centrifuged (10 min, 13k rpm). The pellet was re-suspended in 1 mL methanol/acetone (2:1) and incubated at 68 °C for 20 min. After removing the cell debris following centrifugation for 5 min at 13k rpm, the absorptions (E, extinctions) were measured at 650 nm, 665 nm, and 473 nm with a Spectronic Genesys 20 (Thermo Fisher Scientific) photometer. Calculation of the pigment concentration [69] was as follows: chlorophyll *a*, 11.24 x E_{665nm} – 2.04 x E_{650nm} (µg mL^{-1}); chlorophyll **b**, 20.13 x E_{650nm} – 4.19 x E_{665nm} (µg mL^{-1}); carotenoids total: (1000 x E_{473nm} - 1.9 x chl.*a* - 63.14 x chl. *b*) / 214 (µg mL^{-1}). Also lipid levels were measured following the analyses of lipids as described previously [70, 71]. For the estimation of fatty acids as methyl esters (FAMEs), 1 ml of a methanolic solution containing 2.75 % (v/v) H_2SO_4 (95-97 %) and 2 % (v/v) dimethoxypropane was added to 10 mg lyophilized algae culture. For later quantification of the fatty acids, 100 µg of triheptadecanoate was added, and the sample was incubated for one hour at 80 °C. To extract the resulting FAMEs, 1.5 ml of saturated aqueous NaCl solution and 1.2 ml of hexane were added and centrifuged at 450 g for 10 min. The hexane phase was dried under streaming nitrogen and redissolved in 0.1 ml acetonitrile. GC analysis was performed with an Agilent (Waldbronn, Germany) 6890 gas chromatograph fitted with a capillary DB-23 column (30 m x 0.25 mm; 0.25 µm coating thickness; J&W Scientific, Agilent, Waldbronn, Germany). Helium was used as carrier gas at a 1 ml/min flow rate. The temperature gradient was 150 °C for 1 min, 1–0 - 200 °C at 4 K min^{-1}, 200-250 °C at 5 K $min^{-1,}$ and 250 °C for 6 min. Peak areas were collected with the ChemStation software (Agilent, Waldbronn, Germany).

Statistical analysis

The data were analyzed and visualized using R version 4.1.3 [72], libraries from the tidy verse 1.3.1 package [73], and the ggplot2 package [74].

Abbreviations

CO_2: carbon dioxide; SAG: culture collection of algae at Göttingen University, Germany; PUFAs: polyunsaturated fatty acids; FAMEs: fatty acid methyl esters.

Declarations

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Authors' contributions

Rudolf Tischner and Thomas Friedl conceived and designed the study. Robert Armon developed general aspects concerning the effects of elevated CO_2 on microalgal productivity. Anastasiia Kryvenda selected the test strains, established new algal isolates for the study, and drafted the manuscript. Anastasiia Kryvenda carried out the growth experiments under elevated CO_2 atmospheres. Bastian Steudel assisted with the selection of strains. Carola Griehl performed the carotenoid analyses and critically revised the manuscript. Rudolf Tischner and Thomas Friedl wrote the final manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Figures

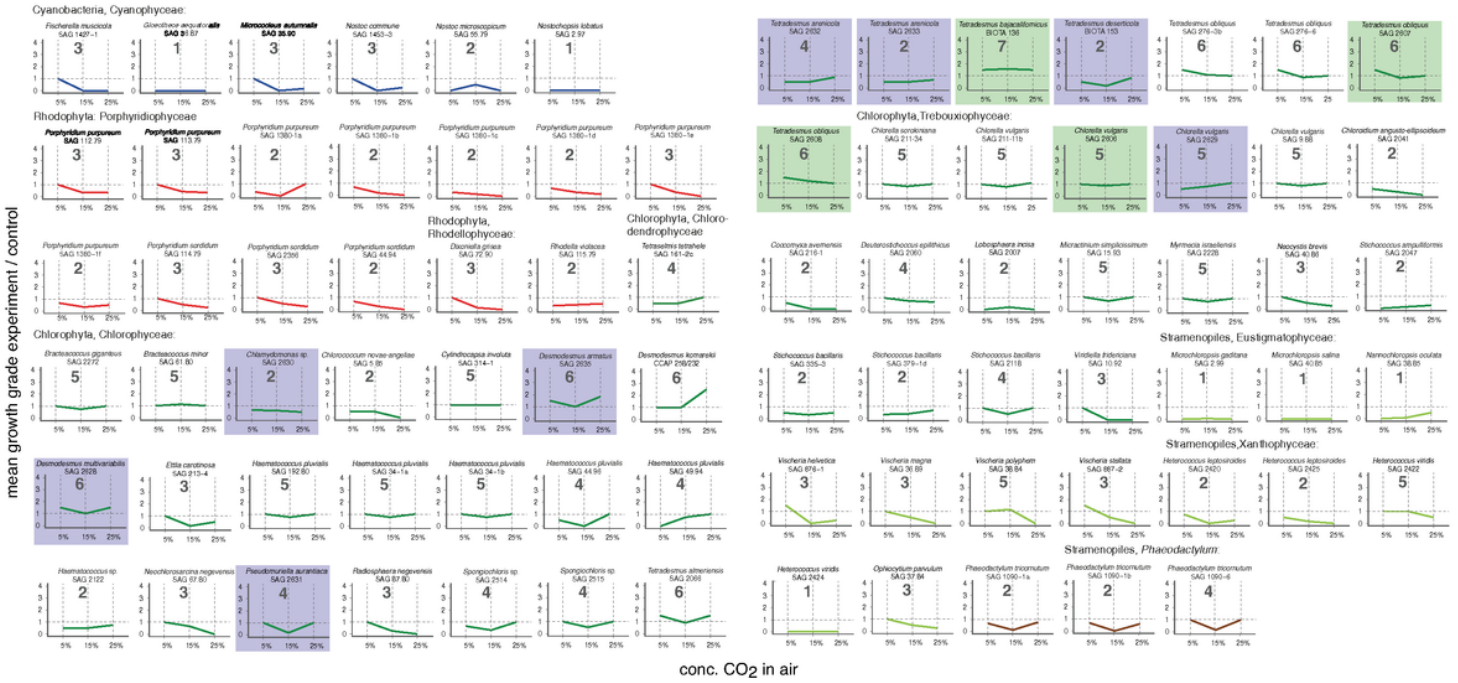

Figure 1

Diagrams showing the mean growth of 81 selected algal strains under atmospheres of elevated CO_2 concentrations in air in relation to controls under ambient CO_2. 1, no change; >1, enhanced growth; <1 decreased growth; blue diagrams, the 12 new terrestrial green algal isolates (Table 1); green, diagrams of the four strains selected for further testing (see text). Bold numbers, general patterns of mean growth: 1, ceased growth under all elevated CO_2 levels; 2, unaffected growth only under 5% CO_2 atmosphere; 3, unaffected growth under 5 %, suppressed or ceased growth under higher levels; 4, negatively affected growth under CO_2 < 25 %, but tolerant to 25 % CO_2; 5, almost unaffected growth at all three levels; 6, enhanced growth at 5% CO_2, almost unaffected at the other levels; 7, enhanced growth under 15 % CO_2 and/or 25 % CO_2 atmospheres.

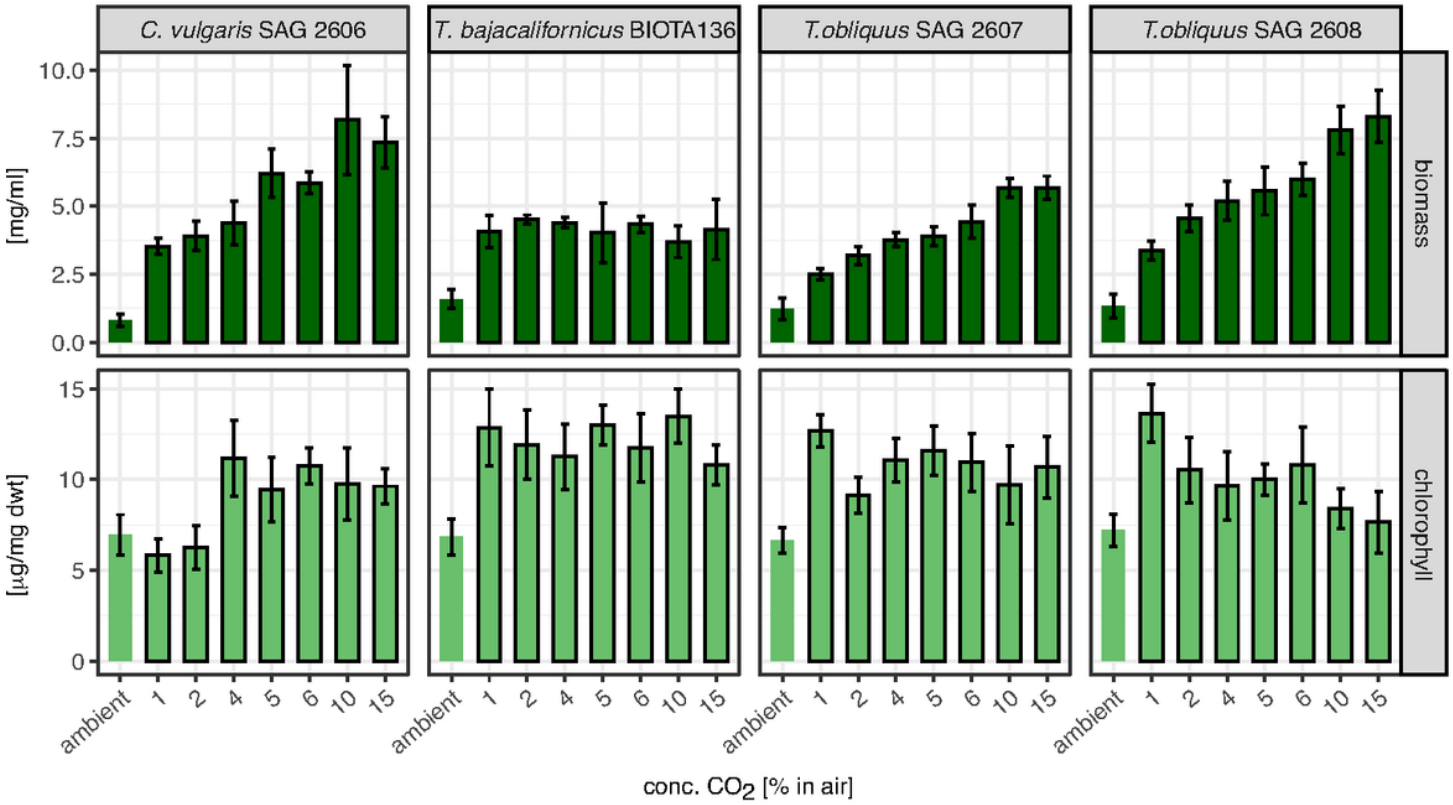

Figure 2

Biomass and chlorophyll productivity of the four selected new terrestrial green algal strains in submerged culture with aeration of increasing CO_2 concentrations in air.

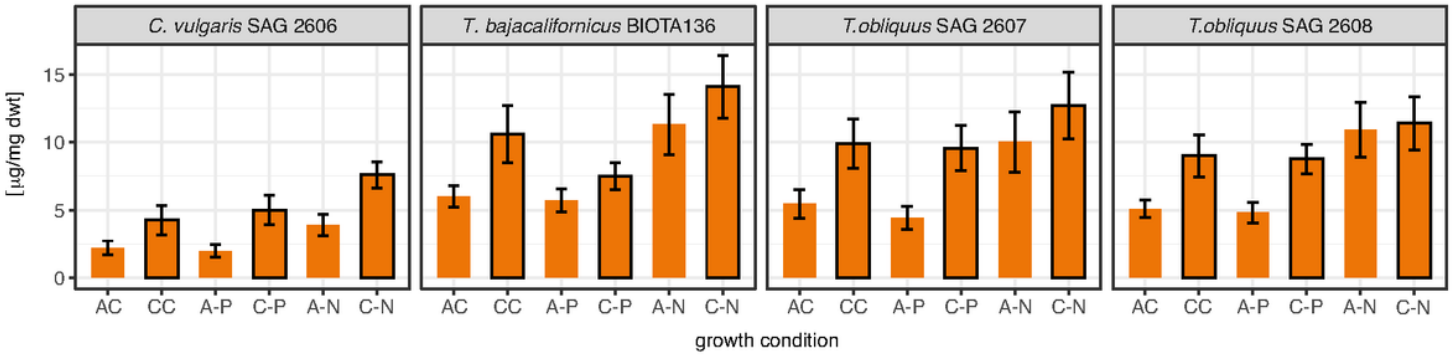

Figure 3

Contents of carotenoids of the four selected terrestrial new green algal strains in full and nutrient-limited growth medium, at ambient or 15% CO_2 aeration in submerged culture. Growth conditions: AC, complete liquid medium aerated with ambient CO_2; CC, complete medium and aeration at 15% CO_2; A-P and C-P, aeration with ambient and 15% CO_2 at phosphate limitation; A-N and C-N, aeration with ambient and 15% CO_2 at nitrogen limitation.

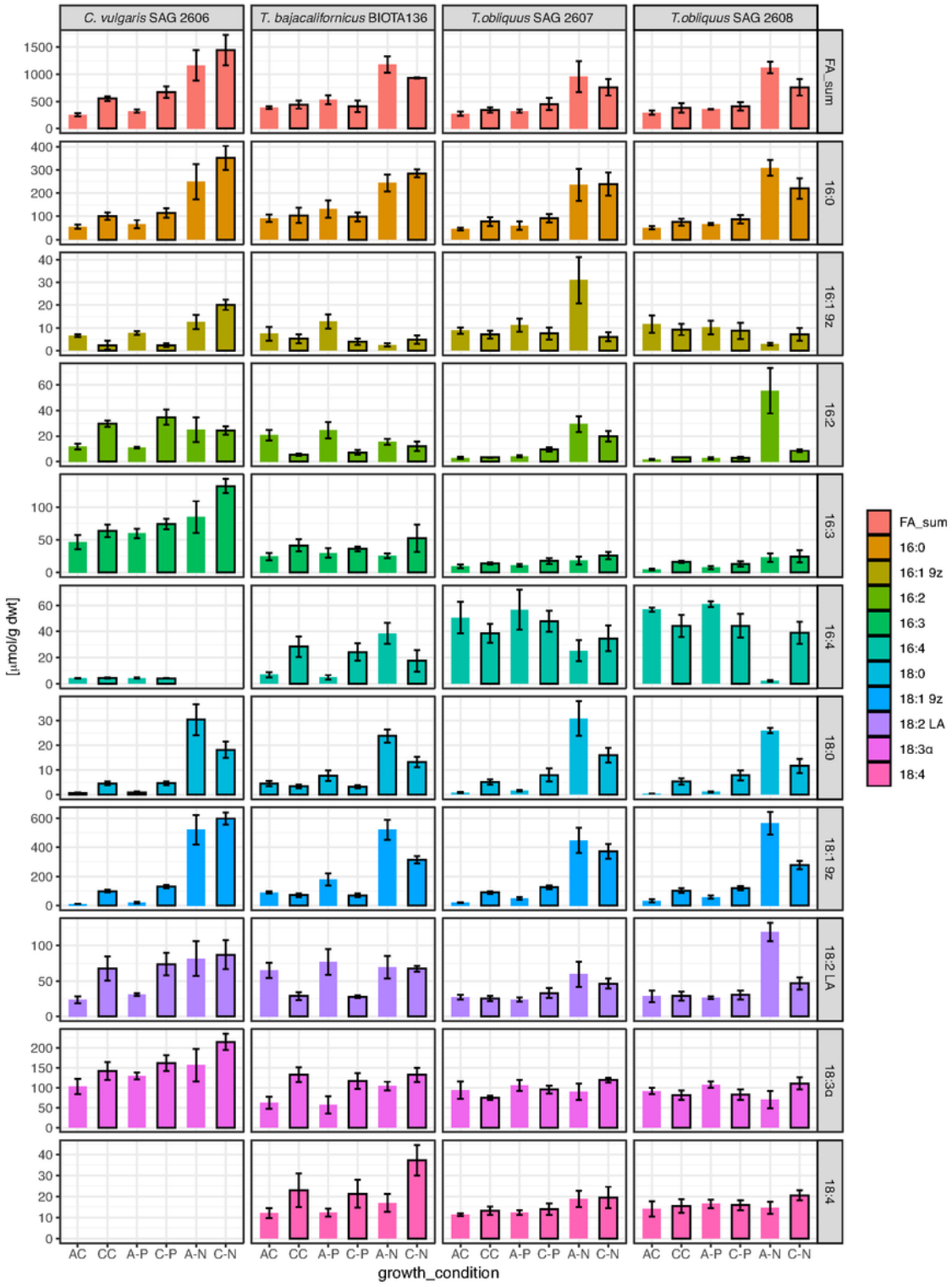

Figure 4

Total fatty acids (FA_sum) and contents of 10 selected fatty acids of the four selected terrestrial new green algal strains in full and nutrient-limited growth medium, at ambient or 15% CO_2 aeration in submerged culture. Growth conditions: AC, complete liquid medium aerated with ambient CO_2; CC,

complete medium and aeration at 15% CO_2; A-P and C-P, aeration with ambient and 15% CO_2 at phosphate limitation; A-N and C-N, aeration with ambient and 15% CO_2 at nitrogen limitation.

Supplementary Files

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