Effects of auxin derivatives on phenotypic plasticity and stress tolerance in the alga *Desmodesmus* (Chlorophyceae, Chlorophyta) (#42400)

First submission

Guidance from your Editor

Please submit by **18 Nov 2019** for the benefit of the authors (and your $200 publishing discount) .

Structure and Criteria
Please read the 'Structure and Criteria' page for general guidance.

Raw data check
Review the raw data. Download from the materials page.

Image check
Check that figures and images have not been inappropriately manipulated.

Privacy reminder: If uploading an annotated PDF, remove identifiable information to remain anonymous.

Files
Download and review all files from the materials page.

11 Figure file(s)
1 Raw data file(s)

Structure and Criteria

Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. **BASIC REPORTING**
2. **EXPERIMENTAL DESIGN**
3. **VALIDITY OF THE FINDINGS**
4. General comments
5. Confidential notes to the editor

You can also annotate this PDF and upload it as part of your review

When ready submit online.

Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your guidance page.

BASIC REPORTING

- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
- Structure conforms to PeerJ standards, discipline norm, or improved for clarity.
- Figures are relevant, high quality, well labelled & described.
- Raw data supplied (see PeerJ policy).

EXPERIMENTAL DESIGN

- Original primary research within Scope of the journal.
- Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

- Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
- All underlying data have been provided; they are robust, statistically sound, & controlled.
- Speculation is welcome, but should be identified as such.
- Conclusions are well stated, linked to original research question & limited to supporting results.

Standout reviewing tips

The best reviewers use these techniques

Tip	*Example*
Support criticisms with evidence from the text or from other sources	*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*
Give specific suggestions on how to improve the manuscript	*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*
Comment on language and grammar issues	*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 – the current phrasing makes comprehension difficult.*
Organize by importance of the issues, and number your points	*1. Your most important issue* *2. The next most important item* *3. …* *4. The least important points*
Please provide constructive criticism, and avoid personal opinions	*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*
Comment on strengths (as well as weaknesses) of the manuscript	*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

Effects of auxin derivatives on phenotypic plasticity and stress tolerance in the alga *Desmodesmus* (Chlorophyceae, Chlorophyta)

Wei-Jiun Lin [1] , **Han-Chen Ho** [2] , **Sheng-Chang Chu** [1] , **Jui-Yu Chou** [Corresp. 1]

[1] Department of Biology, National Changhua University of Education, Changhua, Taiwan

[2] Department of Anatomy, Tzu Chi University, Hualien, Taiwan

Corresponding Author: Jui-Yu Chou
Email address: jackyjau@cc.ncue.edu.tw

The green microalga *Desmodesmus* is characterized by a high degree of phenotypic plasticity, allowing them to be truly cosmopolitan and withstand environmental fluctuations. This flexibility enables *Desmodesmus* to produce a phenotype–environment match across a range of environments broader than that possible if the phenotypic traits were fixed. Indoles and their derivatives are a well-known crucial class of heterocyclic compounds and are widespread in different species of plants, animals, and microorganisms. Indole-3-acetic acid (IAA) may behave as a signaling molecule in microorganisms, and the physiological cues of IAA may also trigger phenotypic plasticity responses in *Desmodesmus*. In this study, we demonstrated that the changes in colonial morphs of *Desmodesmus* were specific to IAA but not to chemically more stable synthetic auxins, naphthalene-1-acetic acid and 2,4-dichlorophenoxyacetic acid. Moreover, inhibitors of auxin biosynthesis and polar auxin transport inhibited cell division. Notably, different algal species (even different intraspecific stains) exhibited phenotypic plasticity different to that of IAA. Thus, the plasticity involving individual-level heterogeneity in morphological characteristics may be crucial for microalgae to adapt to changing or novel conditions, and IAA treatment potentially increases the tolerance of *Desmodesmus* to several stress conditions. In summary, our results provide circumstantial evidence for the hypothesized role of IAA as a diffusible signal in the communication between the microalga and ~~its associated~~ microorganisms. This information is crucial for elucidation of the role of plant hormones in ~~microalgal~~ ecology.

Effects of auxin derivatives on phenotypic plasticity and stress tolerance in the alga *Desmodesmus* (Chlorophyceae, Chlorophyta)

Wei-Jiun Lin[1], Han-Chen Ho[2], Sheng-Chang Chu[1] & Jui-Yu Chou[1]*
[1]Department of Biology, National Changhua University of Education, Changhua 50007, Taiwan
[2]Department of Anatomy, Tzu Chi University, Hualien 97004, Taiwan

Corresponding author.
Correspondence to Jui-Yu Chou
Tel: +886-4-7232105 Ext. 3426; Fax: 886-4-7211156; Email: jackyjau@cc.ncue.edu.tw

Abstract

~~The g~~green microalga *Desmodesmus* ~~is~~ characterized by a high degree of phenotypic plasticity, allowing them to be truly cosmopolitan and withstand environmental fluctuations. This flexibility enables *Desmodesmus* to produce a phenotype–environment match across a range of environments broader ~~than that possible if the phenotypic traits were fixed~~. Indoles and their derivatives are a well-known crucial class of heterocyclic compounds and are widespread in different species of plants, animals, and microorganisms. Indole-3-acetic acid (IAA) may behave as a signaling molecule in microorganisms, and the physiological cues of IAA may also trigger phenotypic plasticity responses in *Desmodesmus*. In this study, we demonstrated that the changes in colonial morphs of *Desmodesmus* were specific to IAA but not to chemically more stable synthetic auxins, naphthalene-1-acetic acid and 2,4-dichlorophenoxyacetic acid. Moreover, inhibitors of auxin biosynthesis and polar auxin transport inhibited cell division. Notably, different algal species (even different intraspecific stains) exhibited phenotypic plasticity different to that ~~of~~ IAA. Thus, the plasticity involving individual-level heterogeneity in morphological characteristics may be crucial for microalgae to adapt to changing or novel conditions, and IAA treatment potentially increases the tolerance of *Desmodesmus* to several stress conditions. In summary, our results provide circumstantial evidence for the hypothesized role of IAA as a diffusible signal in the communication between the microalga and ~~its associated~~ microorganisms. This information is crucial for elucidation of the role of plant hormones in ~~microalgal~~ ecology.

Keywords Coenobial algae · *Desmodesmus* · Indole derivatives · Microalgae · Phenotypic plasticity

Introduction

Phenotypic plasticity can be broadly defined as the capacity of a single genotype to exhibit variable phenotypes in different environments and implies that a species can conquer diverse environments. A well-known example of phenotypic plasticity is changes in multicelled structures in the coenobial algae. Most studies on phenotypic plasticity in coenobial algae have been conducted considering morphological responses to an abiotic factor. Neustupa and Hodač demonstrated that morphological plasticity of *Pediastrum duplex* var. *duplex* is related to the pH dynamics of freshwater lakes (Neustupa & Hodac, 2005). Peña-Castro et al. also reported the phenotypic plasticity in *Scenedesmus incrassatulus* in response to heavy metal stress (Pena-Castro et al., 2004). However, microalgae are typically associated with other microorganisms, such as zooplankton, fungi, and bacteria. Thus, studies on phenotypic plasticity of the coenobial algae have increased in number and broadened their scope from the ~~initial~~ focus on abiotic factors to ~~that on~~ biotic ones. Hessen and Van Donk first indicated that the presence of the grazing pressure from water flea (*Daphnia magna*) can induce colony formation in *Scenedesmus* algae (Hessen & Van Donk, 1993). Furthermore, Lurling et al. proved that the induced colony formation in the presence of herbivores is considered a strategy more efficient than constitutive defenses under variable grazing risk (Lürling & Van Donk, 1996; Lürling, 2003). Wu et al. further revealed that the number of cells per coenobium of *Scenedesmus* increased with the density of *Daphnia* growth, thus indicating a grazer density–dependent response (Wu et al., 2013).

Auxins, which constitute a class of plant hormones, have previously been suggested to regulate physiological responses and gene expression in microorganisms (Spaepen et al., 2007). Furthermore, indole-3-acetic acid (IAA) is one of the most physiologically active auxins that can be produced by a numerous microbial species (Spaepen et al., 2007; Fu et al., 2015). Furthermore, ~~the~~ phylogenetic analyses have revealed that IAA biosynthetic pathways evolved independently in plants, bacteria, algae, and fungi (Fu et al., 2015). The convergent evolution of IAA production leads to the hypothesis that natural selection might have favored IAA as a widespread physiological code in these microorganisms and their interactions. In natural water bodies, the crucial physical associations and biochemical interactions between microalgae and other microorganisms are generally well recognized (Natrah et al., 2014). Piotrowska-Niczyporuk and Bajguz found that IAA plays a crucial role in the growth and metabolism of *Chlorella vulgaris* during a 72-hour culture period (Piotrowska-Niczyporuk & Bajguz, 2014). Jusoh et al. indicated that IAA can induce changes in oil content, fatty acid profiles, and expression of four genes responsible for fatty acid biosynthesis in *C. vulgaris* at early stationary growth phase. In addition, the significance of these interactions in algal phenotypic plasticity has attracted considerable scientific attention (Lürling & Van Donk, 1996; Lürling & Van Donk, 2000; Lürling 2003). We previously used IAA as a signal molecule in microorganisms to simulate a selection pressures caused by interspecific competition. The results indicated that the mean number of cells per particle of *Desmodesmus opoliensis* and *D. komarekii* decreased gradually as the IAA concentration increased gradually. The proportion of *Desmodesmus* unicells in monocultures increased with IAA concentration. We also demonstrated that these unicells exhibited a lower tendency to sedimentation than did large cells and that shrinkage may facilitate nutrient uptake and light capture (Chung et al., 2018). However, whether other coenobial algal species of *Desmodesmus* use the same strategy to overcome stress remains unknown. Hence, the objective of the present study was to compare the effects of IAA at different concentrations on phenotypic responses in different *Desmodesmus* species. Moreover, to address the auxin specificity of these processes and obtain an insight into the complex auxin-related regulatory mechanism(s) in algal physiology, we have selected a group of compounds called "auxin analogs," such as synthetically produced naphthalene-1-acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D), which are structurally related to IAA. We thus aim to determine the differential effects of auxins and auxin-like compounds on the morphological responses of these coenobial algae. ~~Furthermore, IAA has been detected in some species of Scenedesmaceae microalgae (Mazur et al., 2001; Prieto et al., 2011).~~ Therefore, we investigated the effects of inhibitor of auxin biosynthesis and auxin transport in *Desmodesmus*. To elucidate the physiological changes induced by phytohormone treatment, we also investigated whether IAA pretreatment promotes an enhanced stress-tolerant phenotype. The obtained results are crucial for elucidation of the role plant hormones in microalgal physiology.

Materials and Methods

Isolation and Culture of Microalgae

The algal strains used here were isolated from natural water bodies in Central Taiwan. Water samples with visible microalgal population were centrifuged at 3000 ×*g* for 10 minutes at room temperature to concentrate the cells and spread onto CA agar plates. For isolating an axenic single colony from field water samples, the streak plate method was used. The algae were cultured in CA medium, consisting of 2 mg/L $Ca(NO_3)_2.4H_2O$, 10 mg/L KNO_3, 5 mg/L NH_4NO_3, 3 mg/L β–Na_2glycerophosphate.$5H_2O$, 2 mg/L $MgSO_4.7H_2O$, 0.01 μg/L vitamin B12, 0.01 μg/L biotin, 1 μg/L thiamine HCl, and 0.1 mL/L PIV metals (1000 mg/L $Na_2EDTA.2H_2O$, 196 mg/L $FeCl_3.6H_2O$, 36 mg/L $MnCl_2.4H_2O$, 10.4 mg/L $ZnCl_2$, 4 mg/L of $CoCl_2.6H_2O$, and 2.5 mg/L of $Na_2MoO_4.2H_2O$), 0.1 mL/L Fe (as EDTA; 1:1 molar; 702 mg/L $Fe(NH_4)_2(SO_4).6H_2O$ and 660 mg/L $Na_2EDTA.2H_2O$), and 40 mg/L of HEPES; the pH was then adjusted to 7.2. Isolated algal cells were stored at −80°C in 15%–20% glycerol. For each experiment, the alga was cultured axenically in liquid CA medium at 125 rpm in a tube rotator and grown at 25°C under cool white fluorescent light (approximately 46.30 μmol m^{-2} s^{-1}) with a 14:10-h light–dark period.

Algae Identification

The algal cells were harvested by centrifugation at 3000 ×*g*~~,~~ at 25°C for 10 minutes. The genomic DNA used for analysis was isolated using AccuPrep GMO DNA Extraction Kit (Bioneer, Korea). The 18S rDNA was amplified through PCR by using the following primers: 18S forward-TTTCTGCCCTATCAACTTTCGATG and 18S reverse-TACAAAGGGCAGGGACGTAAT, which yielded a fragment of approximately 1200 bp (Pan et al., 2011). The PCR conditions were as follows: initial denaturation at 96°C for 4 minutes; 36 cycles of denaturation at 96°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 minutes; and final extension at 72°C for 6 minutes. The ITS1-5.8 S-ITS2 ~~region~~ rDNA was amplified using the primers ITS forward1 (ACCTAGAGGAAGGAGAAGTCGTAA) and ITS reverse1 (TTCCTCCGCTTATTGATATGC), which provided a fragment of approximately 1200 bp (Pan et al., 2011). The PCR conditions ~~are~~ as follows: initial denaturation at 96°C for 4 minutes; 36 cycles of denaturation at 96°C for 30 s, annealing at 48°C for 30 s, and extension at 72°C for 1 minutes; and final extension at 72°C for 6 minutes. DNA sequencing was performed by Tri-I Biotech, Inc. The Basic Local Alignment Search Tool was used to find regions of local similarity between sequences on the website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Experimental Design

Solutions containing different concentrations of phytohormones (IAA, NAA, and 2,4-D) and auxin-related compounds (4-biphenylboronic acid and 2,3,5-triiodobenzoic acid) were prepared to investigate their influence on the growth and morphological plasticity of *Desmodesmus*. The concentrations of each phytohormone and compounds used in each experiment depended on the sensitivity of each species. Algae were harvested after each experiment, and the proportions of different-celled populations were calculated under an optical microscope (DMRB, Leica, Germany). The proportion of different algal populations (including unicellular; two-, four-, and eight-celled; and other colonial morphs) were calculated, and the mean numbers of cells in different morphotypes were calculated. The numbers of cells per coenobium were counted by dividing the total cell number by the number of coenobia.

Transmission Electron Microscopy

All specimens were prefixed in 2.5% glutaraldehyde/0.1 M sodium cacodylate buffer (pH 7.3) containing 1% tannic acid at 4°C overnight. After in 0.1 M sodium cacodylate buffer with 5% sucrose for 15 minutes ~~were washed~~ three times, specimens were postfixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer at 4°C overnight. Specimens were then washed in buffer, *en bloc* stained with 2% aqueous uranyl acetate, dehydrated through a graded series of ethanol and two times with 100% acetone. Specimens were infiltrated with Spurr resin overnight and embedded in fresh Spurr resin the next day. Serial ultrathin sections of approximately 70 nm were cut with a diamond knife on a Leica Ultracut R ultramicrotome (Leica, Heerbrugg, Switzerland) and examined with a Hitachi H-7500 transmission electron microscope (Hitachi, Tokyo, Japan) at 80 kV. Images were recorded using a 2048 × 2048 Macrofire monochrome CCD camera (Optronics, Goleta, CA, USA).

Stress Tests

The culture samples were harvested; the cells were then washed with CA medium and resuspended in the CA medium with different treatments. For osmotic shock test, the cells were incubated in the CA medium with 0.5 M NaCl. For the acidic pH assay, the culture samples were resuspended in CA medium at pH 3.0 (adjusted with HCl). For oxidative stress, the cells were exposed to hydrogen peroxide at final concentration of 5 mM. The cell suspensions subjected to the aforementioned treatments were shaken at 25°C for 15 or 30 minutes. For inducing heat shock, the cells were exposed to 40°C for 10, 15, or 20 minutes by immersing the cultures in a shaking water bath. For cold treatment, the cultures were exposed to 4°C for 24 hours. Fractions of viable cells of each experiment were determined by plating appropriate dilutions of the cultures on CA agar plates before and after treatments. The controls (without IAA treatment) received the same treatments used throughout the procedure.

Statistical Analysis.

Data are presented as means of three replicates ± their standard deviations (SDs). The proportions of colonies with different numbers of cells and mean number of cells per coenobium were compared using a one-way analysis of variance (ANOVA) with least significant difference post hoc test. In stress tests, the significance of differences between the groups was determined using the Student t test and ANOVA. A p of <0.05 was considered statistically significant.

Results

Effect of Auxin Analogs on Algal Growth and Phenotypic Plasticity Induction

In a previous study, we performed a dose–response analysis to determine the fitness effects of IAA on the coenobial algae *D. komarekii* (Chung et al., 2018). The results revealed that different concentrations of IAA had different effects on the growth and morphological changes of *D. komarekii*. Thus, we concluded that *Desmodesmus* can respond to the external phytohormone IAA signal and then integrate the information to initiate physiological changes. In this study, our aim was to determine whether the physiological cues of IAA-related compounds also trigger the growth and phenotypic plasticity responses in *Desmodesmus*. With respect to *D. komarekii* growth, we examined the effects of the natural auxin IAA as well as those of the synthetic auxins NAA and 2,4-D. At 300 µM, IAA, NAA, and 2,4-D clearly inhibited growth; however, IAA caused lower inhibition than did NAA and 2,4-D (Fig. 1a). This inhibitory effect was also observed at 100 and 200 µM NAA and 2,4-D, but not in the cells treated with 100 µM IAA (Fig. 1b, c). These observations indicated that these auxin-related compounds inhibit *D. komarekii* growth.

To measure phenotypic plasticity responses in algal populations, monocultures of *D. komarekii* were used. After 1 week of culturing, the monocultures of *D. komarekii* in the groups with exogenous 300 µM IAA and synthetic auxins were compared with those in the control environment (without treatment). We found that the monocultures of *D. komarekii* in the control groups (without IAA treatment) were dominated by one- and four-celled coenobia (Fig. 2a–c). The morphology of *D. komarekii* monocultures changed drastically compared with the control after exposure to IAA (Fig. 2b, c). The proportion of unicells increased rapidly from day 3, and the proportion of four-celled coenobia decreased (Fig. 2a, c). The mean number of cells per particle reached its minimum on approximately day 7 (Fig. 2a), the proportion of unicells increased from 37% to approximately 73%, and the proportion of four-celled coenobia decreased from 49% to approximately 16% on day 9. The proportion of two-celled coenobia changed only slightly from approximately 13% to approximately 7%. The mean number of cells per particle in the control groups remained at >2.5 during the 9-day period. In this experiment, the *D. komarekii* population of each culture was composed of unicells and two-, four-, and eight-celled colonies; a few three-, five-, six-, and seven-celled colonies were also present, but coenobia with more than eight cells were not observed.

Through transmission electron microscopy (TEM) analysis, we confirmed that the morphological changes in coenobia were not caused by cell aggregation but by the vegetative growth of a mother cell (Fig. 3a). No extracellular matrix was seen on or around the cells, and the connecting strands between cells were highly visible (Fig. 3b). Dense section of warty layer can be seen over each coenobial junction (yellow circle; Fig. 3c). Notably, we observed that specific large unicells were formed in the monocultures of *D. komarekii* after day 5 under IAA treatment. Thus, the samples collected at day 7 after IAA treatment and the cells in the control groups were used for observation of morphology through TEM. The accumulation of many starch granules and lipid bodies was observed in the large unicells compared with the cells in control groups (Fig. 3d). By contrast, we found that the auxin-related compounds NAA and 2,4-D both inhibit the growth of *D. komarekii* in dose-dependent manner, but they did not influence their number of cells of individual (Fig. 2d, e). We next tested the effects of an auxin biosynthesis inhibitors and a polar auxin transport inhibitor. Here, 4-biphenylboronic acid (BBo), a potent YUCCA enzyme inhibitor and *Arabidopsis* growth inhibitor, and 2,3,5-triiodobenzoic acid (TIBA), a polar auxin transport inhibitor, were used [(Dhonukshe et al. 2008; Kakei et al. 2015)]. BBo strongly inhibited growth even at 100 µM and its inhibitory effect increased with its concentration (Fig. 1c). At 200 and 300 µM, both of TIBA and BBo inhibited *D. komarekii* growth (Fig. 1a, b). These results suggested that inhibition of auxin transport and inhibition of YUCCA function both inhibit cell growth.

The auxin-like physiological competence of selected compounds was analyzed in *Desmodesmus* based on the inhibition of growth in liquid cultures and morphological changes. Thus, we performed a dose–response analysis to determine the fitness effects of IAA and other analogs on eight other *Desmodesmus* strains. The results revealed that different concentrations of indole derivatives had divergent effects on the growth of different *Desmodesmus* species (Figs. 4–11). In general, high concentrations (>300 µM) of IAA and other analogs inhibited the growth of the algal population. Thus, *Desmodesmus* can respond to the external phytohormone signal of IAA and other analogs and then integrate the information to initiate physiological changes. In the subsequent experiment, our aim was to determine whether the physiological cues of IAA and other analogs in these cultures also trigger phenotypic plasticity responses.

Plastic Phenotypic Changes in Response to IAA Are Strain-Dependent Behaviors

To measure the phenotypic plasticity responses to indole derivatives on four strain of *D. armatus*, two strains of *D. communis*, and one strain each of *D. intermedius* and *D. opoliensis* were used in this study. After 1 week of treatment, the monocultures of *D. armatus* in the control groups (without indole derivative~~s~~ treatment) were compared with indole derivatives. We found that the changes in colonial morphs in *D. armatus* are specific to IAA but not to chemically to synthetic auxins, NAA and 2,4-D, which are chemically more stable than IAA (Figs. 4–6). Moreover, we found the different algal strains of *D. armatus* demonstrat~~ing~~ phenotypic plasticity ~~different~~ to ~~IAA~~. In *D. armatus* JYCA037, the monocultures in the control groups were dominated by two- and four-celled coenobia, with <2% unicells (Fig. 4a, b). The morphology of *D. armatus* JYCA037 populations considerably changed under high concentration of IAA treatment compared with that in the control environment (without IAA addition). When the IAA concentration increased, the proportion of four-celled coenobia declined from >90% to approximately 21%, and the number of unicells increased from <2% to approximately 12% and two-celled coenobia increased from approximately 7% to 66%. The mean number of cells per particle of *D. armatus* JYCA037 decreased gradually as the IAA concentration gradually increased, and the cell number reached its minimum level at an IAA concentration of 400 µM (Fig. 4a). Similar results were observed in the monocultures of *D. communis* JYCA040; it was dominated by two- and four-celled coenobia in the control groups (Fig. 8). When IAA concentration increased, the proportion four-celled coenobia decreased, and the number of unicells increased. The mean number of cells per coenobium particle in the control groups of these two strains remained for >3 days after 7-day culturing. By contrast, in *D. armatus* JYCA041, the monocultures in the control groups were dominated by unicells (47%), with 47% two- and <7% four-celled individuals (Fig. 4c, d). The morphology of *D. armatus* JYCA041 populations changed considerably under high concentration of IAA treatment compared with that in the control environment (without IAA addition). When IAA concentration increased, the proportion of two-celled coenobia increased from approximately 47% to approximately 69% and the number of unicells declined from approximately 47% to approximately 28%. The proportion of four-celled coenobia only slightly changed from approximately 6% to approximately 3%. The mean number of cells per particle of *D. armatus* JYCA041 increased gradually as the IAA concentration gradually increased and reached its maximum level at an IAA concentration of 400 μM (Fig. 4b) Similarly, the mean number of cells per particle of *D. armatus* JYCA039 increased gradually as the IAA concentration gradually increased and reached its maximum level when the IAA concentration was approximately 200 μM (Fig. 7a, b). Notably, the aformentioned morphological changes were not observed in *D. armatus* JYCA045 even under treatment with high concentrations with IAA (Fig. 4e, f). By contrast, we found that the auxin-related compounds NAA and 2,4-D both inhibit *Desmodesmus* growth in a dose-dependent manner, but the treatment did not influence their number of cells of individuals in these four *D. armatus* strains (Figs. 4 and 5). The strain-dependent response to IAA but not to NAA and 2,4-D also occurred in one strain of *D. communis* (JYCA040; Fig. 8) and *D. opoliensis* (JYCA043; Fig. 9). However, the phenotypic plasticity caused by auxin analogs was not obviously shown in ~~one~~ strain of *D. communis* (JYCA044; Fig. 10) and *D. intermedius* (JYCA042; Fig. 11).

IAA Improves Algal Defenses Against Stress

In this study, we found that starch granules and lipid bodies accumulated in algal cells grown at a high IAA concentration. In this environment, algal cells also demonstrated slow growth. Thus, algae contain storage in the form of natural oils, such as neutral lipids or triglycerides, and algal growth diminishes when exposed to stresses. Thus, we propose that the morphological responses and the associated physiological changes provide some fitness advantages to *Desmodesmus*, such as the ability to survive in the water bodies often exposed to fluctuations in environmental factors. The data reported in Table 1 showed that IAA-treated cells could withstand sudden changes in the environment, demonstrating significantly longer survival rates in the media subjected to temperature shock (40°C, 15 minutes and 4°C, 24 hours) and acid treatment (pH 3.0, 15 minutes). The data also shows that IAA treatment also marginally but significantly increased the survival rate of microalgae treated with 5 mM H_2O_2 for 30 minutes (p = 0.052). However, although the survival rates did not increase significantly, the average survival rates of IAA-treated cells were higher than those of the controls in many treatments.

Discussion

A central orienting question in biodiversity theory and ecology is the "paradox of the plankton," which indicates that the number of coexisting planktonic species far exceeds the expected and explicable number based on competition theory (Hutchinson, 1961). Ecologists have provided multiple solutions to the paradox by applying game theory, chaos, tradeoffs, and many other concepts in the past five decades (Tilman, 1994; Huisman & Weissing, 1999; Károlyi et al., 2000; Kerr et al., 2002; Goyal & Maslov, 2018). A leading theory to explain the paradox is that individual variability maintains high biodiversity in planktonic microorganisms (Menden-Deuer & Rowlett, 2014). In aquatic ecosystems, significant evidence supports individual variability, in individual behaviors or physiology, among planktonic microorganisms. This phenotypic plasticity has played a central role in studies on the evolution of diversity. Ecologically, phenotypic plasticity has been considered particularly crucial when environmental changes occur and different phenotypes have different fitness values across environments that decide the survival an individual in the face of environmental changes (West-Eberhard, 1989). The plasticity even can potentiate evolvability of microorganisms by opening up new regions of the adaptive landscape (Yi & Dean, 2016).

In our study, we revealed that the morphological characteristics of *Desmodesmus* changed considerably when exposed to IAA compared with the algal cells in the control environment. We found that the algal strains we assayed here have different response patterns to the external IAA. In this study and our previous study, we found that when IAA concentration increased, the mean number of cells per particle of some *Desmodesmus* species decreased (Chung et al., 2018). The surface-to-volume ratios of the unicells was larger than the colony cells in microalgae. Previous literatures have reported that the changes in colony size influence algal surface-to-volume ratios, and the surface-to-volume ratio can affect light capture and nutrient uptake (Reynolds, 2006; Steele et al., 2009). Notably, in this study, we found that in some algal strains, this trend was reversed: the mean number of cells per particle of some *Desmodesmus* strains was increased when IAA concentration increased. These colonial populations have higher sinking velocities than the unicells and two-celled coenobia. The colonial populations have higher sinking velocities than unicellular cells; consequently, their competitive ability of microalgae might be altered (Lürling, 2003). Thus, plasticity involving individual-level heterogeneity in behaviors and physiological characteristics is crucial for planktonic microorganisms to adapt to changing or novel conditions. This may suggest that individual variability is perhaps the key mechanism supporting planktonic biodiversity.

In this study, two widely used auxins in plant tissue culture, NAA and 2,4-D, were also used to investigate their effect on algal growth and physiological responses. These synthetic auxins show varying degrees of auxin-like activity in different bioassays (Abebie et al., 2007; Savaldi-Goldstein et al., 2008). For instance, in Simon et al., the seedlings of *Arabidopsis thaliana* and suspension-cultured cells of *Nicotiana tabacum* BY-2 were used to investigate the physiological activity of several auxin analogs, along with their capacity to induce auxin-dependent gene expression, to inhibit endocytosis and to be transported across the plasma membrane (Simon et al., 2013). The authors concluded that the major determinants for the auxin-like physiological potential of a particular compound are highly complex and involve its chemical and metabolic stability, its ability to distribute in tissues in a polar manner, and its activity toward auxin-signaling machinery. Thus, the distinct behavior of some synthetic auxin analogs suggests that they might be useful tools in investigations of the molecular mechanism of auxin action. Ohtaka et al. also examined the responses of the natural auxin (indole-3-butylic acid; IBA) as well as the synthetic auxins (NAA and 2,4-D) on the charophyte alga *Klebsormidium nitens* (Ohtaka et al., 2017). Consistent with our results, the authors indicated that these auxin-related compounds all inhibit *K. nitens* growth in a dose-dependent manner. Notably, the IAA was detected in cultures of *K. nitens*, but *K. nitens* lacks the central regulators of the canonical auxin-signaling pathway found in land plants. However, the authors found that the exogenous IAA inhibited cell division and elongation, and this treatment rapidly induced expression of the transcription factor lateral organ boundaries-domain. During evolution, *K. nitens* may have acquired a primitive auxin-response pathway to regulate transcription and cell growth. Here, we found that the natural auxin IAA and the synthetic auxins NAA and 2,4-D can all influence *Desmodesmus* growth rate. However, the changes in the colonial morphs in *Desmodesmus* are specific to IAA, but not to chemically more stable synthetic auxins. These studies have suggested that structure–activity relationships determined precisely at the level of a particular protein (e.g., receptor or carrier) may not correspond completely to the final auxin-like physiological activity of a particular compound in the streptophytes and their sister group, the chlorophytes. Thus, the comparison of the structure–activity relationships for the aforementioned phenotypic changes highlights differences in the structural requirements of these auxin-related physiological processes, thus making the differential (or the same) phenotypic outcome of the same (or different) compound a very crucial aspect of auxin biology.

Microalgae are unicellular photosynthetic microorganisms, typically found in freshwater and marine systems. The high flexibility and adaptability of this extremely diverse group of eukaryotic organisms enable it to grow in diverse environments, including fresh saltwater, blackish, marine, and soil environments. These coexist with heterotrophic microorganisms, and the exchange of chemical compounds is central to the interactions of microalgae with other microorganisms. How microalgal–microbial interactions and participating chemical compounds shape their communities and considerably affect their fitness remains unknown (Hom et al., 2015). Notably, not only plants but also bacteria, fungi (including yeast), and even some microalgae produce or respond to IAA (Fu et al., 2015). Researchers have hypothesized that the microbes sense environmental IAA concentrations to determine the cell density of its competitors (Spaepen et al., 2007; Fu et al., 2015; Chung et al., 2018). Thus, IAA has been speculated to be a signal that coordinates microbial behavior to enhance protection against damage by adverse conditions (Bianco et al., 2006; Chung et al., 2017). Here, we confirmed that the physiological changes in response to IAA confers a fitness advantage by promoting the ability of *Desmodesmus* to survive in their niches that often undergo fluctuations in environmental factors, such as temperature, osmotic pressure, reactive oxygen species, and pH changes. Under unfavorable stress conditions, such as nutritional starvation, salinity stress and high light intensity, lipid production is usually enhanced in algal cells, due to shifts in lipid biosynthetic pathways toward neutral lipid accumulation (Sun et al., 2018). Microalgae generally accumulate neutral lipids, mainly in the form of triacylglycerols (TAG) under environmental stress conditions. The accumulation of TAG likely occurs as a means of creating an energy deposit that can be readily used in response to a more favorable environment allowing for rapid growth (Tan & Lee, 2016). In green algae, stress conditions also trigger the accumulation of starch granules in the cells, with starch accumulation preceding the accumulation of lipid bodies following stress onset (Siaut et al., 2011). It is generally assumed that the starch and TAG serve as electron sinks under conditions where photosynthesis or metabolism of an exogenous carbon source remains active but the growth is limited (Hu et al., 2008). This phenomenon suggests that carbon sources in algal cells during stress conditions were allocated ~~to~~ not only storage lipid production but also starch biosynthesis, and this finding demonstrates the possibility of partitioning manipulation in the cells. To link physiological changes to phenotype, we performed various cell viability assays in response to heat, cold, osmotic stress, oxidative stress, reactive oxygen species, and pH changes. We found an increased ability to tolerate these stresses, thereby confirming the inferred enhanced stress-tolerant phenotypes when exposed to IAA. The results are consistent with earlier research ~~that~~ on bacteria ~~and~~ found enhanced stress tolerance when the bacteria were pretreated with IAA across various stress conditions (Bianco et al., 2006; Imperlini et al. 2009; Donati et al., 2013).

In natural water bodies, the importance of physical associations and biochemical interactions between microalgae and microorganisms is generally well appreciated, but the significance of these interactions to microbial ecology has not been investigated. In our previous study, we found that a low concentration of IAA promoted the growth of algal cells, but high concentrations of IAA inhibited cell growth (Chung et al., 2018). Herein, we further proved that the effects of exogenous IAA and on algal growth and phenotypic changes is species- and even strain-dependent. IAA can exert stimulatory and inhibitory effects on not only algae, fungi, and yeast but also bacteria (Prusty et al., 2004; De-Bashan et al., 2008; Hu et al., 2010; Kerkar et al. 2012; Kulkarni et al., 2013; Sun et al., 2014; Liu et al., 2016; Fu et al., 2017). Bagwell et al. reported the frequency of co-occurrence between IAA-producing bacteria and green algae in natural and engineered ecosystems and revealed that the chlorophyll content and dry weight of algal cells were IAA concentration-dependent responses (Bagwell et al., 2014). A recent study also indicated that IAA produced by associated bacteria was transferred to diatom and influence their growth in exchange for organosulfur compounds (Amin et al., 2015). Thus, exposure to IAA could be likely to affect the outcome of competition among these coexisting organisms. We finally suggested that both algae and microorganisms altered their metabolism to defend themselves form their competitors (or suit each other's needs), and this interaction is potentially very prevalent in the aquatic ecosystems. These findings indicated that IAA is a major factor determining the competition (or mutualistic interactions) between microbial species occupying the same niche.

Acknowledgments We thank the members of the Chou Laboratory for their helpful discussions and comments on the manuscript. We are grateful to the staff of the Electron Microscopy Laboratory, Tzu Chi University, for technical support. This manuscript was edited by Wallace Academic Editing. This work was supported by grants from the Ministry of Science and Technology (MOST 105-2311-B-018-001-MY3 and MOST 108-2621-B-018-002-MY3 to J.-Y. Chou).

References

Abebie B, Lers A, Philosoph-Hadas S, Goren R, Riov J, and Meir S. 2007. Differential effects of NAA and 2, 4-D in reducing floret abscission in Cestrum (Cestrum elegans) cut flowers are associated with their differential activation of Aux/IAA homologous genes. *Annals of botany* 101:249-259.

Amin S, Hmelo L, Van Tol H, Durham B, Carlson L, Heal K, Morales R, Berthiaume C, Parker M, and Djunaedi B. 2015. Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* 522:98.

Bagwell CE, Piskorska M, Soule T, Petelos A, and Yeager CM. 2014. A diverse assemblage of indole-3-acetic acid producing bacteria associate with unicellular green algae. *Applied biochemistry and biotechnology* 173:1977-1984.

Bianco C, Imperlini E, Calogero R, Senatore B, Amoresano A, Carpentieri A, Pucci P, and Defez R. 2006. Indole-3-acetic acid improves Escherichia coli's defences to stress. *Archives of Microbiology* 185:373-382.

Chung T-Y, Sun P-F, Kuo J-I, Lee Y-I, Lin C-C, and Chou J-Y. 2017. Zombie ant heads are oriented relative to solar cues. *Fungal Ecology* 25:22-28.

Chung TY, Kuo CY, Lin WJ, Wang WL, and Chou JY. 2018. Indole-3-acetic-acid-induced phenotypic plasticity in Desmodesmus algae. *Sci Rep* 8:10270. 10.1038/s41598-018-28627-z

De-Bashan LE, Antoun H, and Bashan Y. 2008. INVOLVEMENT OF INDOLE-3-ACETIC ACID PRODUCED BY THE GROWTH-PROMOTING BACTERIUM AZOSPIRILLUM SPP. IN PROMOTING GROWTH OF CHLORELLA VULGARIS 1. *Journal of Phycology* 44:938-947.

Dhonukshe P, Grigoriev I, Fischer R, Tominaga M, Robinson DG, Hašek J, Paciorek T, Petrášek J, Seifertová D, and Tejos R. 2008. Auxin transport inhibitors impair vesicle motility and actin cytoskeleton dynamics in diverse eukaryotes. *Proceedings of the National Academy of Sciences* 105:4489-4494.

Donati AJ, Lee H-I, Leveau JH, and Chang W-S. 2013. Effects of indole-3-acetic acid on the transcriptional activities and stress tolerance of Bradyrhizobium japonicum. *PloS one* 8:e76559.

Fu S-F, Chen H-W, Wei J-Y, Lee Y-I, and Chou J-Y. 2017. Yeast-produced IAA is not only involved in the competition among yeasts but also promotes plant growth and development. *Nova Hedwigia* 105:135-150.

Fu SF, Wei JY, Chen HW, Liu YY, Lu HY, and Chou JY. 2015. Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. *Plant Signal Behav* 10:e1048052. 10.1080/15592324.2015.1048052

Goyal A, and Maslov S. 2018. Diversity, stability, and reproducibility in stochastically assembled microbial ecosystems. *Physical review letters* 120:158102.
Hessen DO, and Van Donk E. 1993. Morphological changes in Scenedesmus induced by substances released from Daphnia. *Archiv fur Hydrobiologie* 127:129-129.
Hom EF, Aiyar P, Schaeme D, Mittag M, and Sasso S. 2015. A chemical perspective on microalgal–microbial interactions. *Trends in plant science* 20:689-693.
Hu M, Zhang C, Mu Y, Shen Q, and Feng Y. 2010. Indole affects biofilm formation in bacteria. *Indian J Microbiol* 50:362-368. 10.1007/s12088-011-0142-1
Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, and Darzins A. 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *The plant journal* 54:621-639.
Huisman J, and Weissing FJ. 1999. Biodiversity of plankton by species oscillations and chaos. *Nature* 402:407.
Hutchinson GE. 1961. The paradox of the plankton. *The American Naturalist* 95:137-145.
Imperlini E, Bianco C, Lonardo E, Camerini S, Cermola M, Moschetti G, and Defez R. 2009. Effects of indole-3-acetic acid on Sinorhizobium meliloti survival and on symbiotic nitrogen fixation and stem dry weight production. *Appl Microbiol Biotechnol* 83:727-738. 10.1007/s00253-009-1974-z
Kakei Y, Yamazaki C, Suzuki M, Nakamura A, Sato A, Ishida Y, Kikuchi R, Higashi S, Kokudo Y, and Ishii T. 2015. Small-molecule auxin inhibitors that target YUCCA are powerful tools for studying auxin function. *The plant journal* 84:827-837.
Kerkar S, Raiker L, Tiwari A, Mayilraj S, and Dastager S. 2012. Biofilm-associated indole acetic acid producing bacteria and their impact in the proliferation of biofilm mats in solar salterns. *Biologia* 67:454-460.
Kerr B, Riley MA, Feldman MW, and Bohannan BJ. 2002. Local dispersal promotes biodiversity in a real-life game of rock–paper–scissors. *Nature* 418:171.
Károlyi G, Péntek Á, Scheuring I, Tél T, and Toroczkai Z. 2000. Chaotic flow: the physics of species coexistence. *Proceedings of the National Academy of Sciences* 97:13661-13665.
Kulkarni GB, Sanjeevkumar S, Kirankumar B, Santoshkumar M, and Karegoudar T. 2013. Indole-3-acetic acid biosynthesis in Fusarium delphinoides strain GPK, a causal agent of Wilt in Chickpea. *Applied biochemistry and biotechnology* 169:1292-1305.
Lürling M. 2003. Phenotypic plasticity in the green algae Desmodesmus and Scenedesmus with special reference to the induction of defensive morphology. Annales de Limnologie-International Journal of Limnology: EDP Sciences. p 85-101.
Lürling M, and Van Donk E. 1996. Zooplankton-induced unicell-colony transformation in Scenedesmus acutus and its effect on growth of herbivore Daphnia. *Oecologia* 108:432-

437.

Lürling M, and Van Donk E. 2000. Grazer-induced colony formation in Scenedesmus: are there costs to being colonial? *Oikos* 88:111-118.

Liu Y-Y, Chen H-W, and Chou J-Y. 2016. Variation in indole-3-acetic acid production by wild Saccharomyces cerevisiae and S. paradoxus strains from diverse ecological sources and its effect on growth. *PloS one* 11:e0160524.

Mazur H, Konop A, and Synak R. 2001. Indole-3-acetic acid in the culture medium of two axenic green microalgae. *Journal of applied phycology* 13:35-42.

Menden-Deuer S, and Rowlett J. 2014. Many ways to stay in the game: individual variability maintains high biodiversity in planktonic microorganisms. *Journal of The Royal Society Interface* 11:20140031.

Natrah FM, Bossier P, Sorgeloos P, Yusoff FM, and Defoirdt T. 2014. Significance of microalgal–bacterial interactions for aquaculture. *Reviews in Aquaculture* 6:48-61.

Neustupa J, and Hodac L. 2005. Changes in shape of the coenobial cells of an experimental strain of Pediastrum duplex var. duplex (Chlorophyta) reared at different pHs. *Preslia* 77:439-452.

Ohtaka K, Hori K, Kanno Y, Seo M, and Ohta H. 2017. Primitive auxin response without TIR1 and Aux/IAA in the charophyte alga Klebsormidium nitens. *Plant physiology*:pp. 00274.02017.

Pan Y-Y, Wang S-T, Chuang L-T, Chang Y-W, and Chen C-NN. 2011. Isolation of thermo-tolerant and high lipid content green microalgae: oil accumulation is predominantly controlled by photosystem efficiency during stress treatments in Desmodesmus. *Bioresource technology* 102:10510-10517.

Pena-Castro JM, Martinez-Jeronimo F, Esparza-Garcia F, and Canizares-Villanueva RO. 2004. Phenotypic plasticity in Scenedesmus incrassatulus (Chlorophyceae) in response to heavy metals stress. *Chemosphere* 57:1629-1636. 10.1016/j.chemosphere.2004.06.041

Piotrowska-Niczyporuk A, and Bajguz A. 2014. The effect of natural and synthetic auxins on the growth, metabolite content and antioxidant response of green alga Chlorella vulgaris (Trebouxiophyceae). *Plant growth regulation* 73:57-66.

Prieto C, Rosa E, Cordoba C, Nancy M, Montenegro J, Andres M, and González-Mariño GE. 2011. Production of indole-3-acetic acid in the culture medium of microalga Scenedesmus obliquus (UTEX 393). *Journal of the Brazilian Chemical Society* 22:2355-2361.

Prusty R, Grisafi P, and Fink GR. 2004. The plant hormone indoleacetic acid induces invasive growth in Saccharomyces cerevisiae. *Proceedings of the National Academy of Sciences* 101:4153-4157.

Reynolds CS. 2006. *The ecology of phytoplankton*: Cambridge University Press.
Savaldi-Goldstein S, Baiga TJ, Pojer F, Dabi T, Butterfield C, Parry G, Santner A, Dharmasiri N, Tao Y, and Estelle M. 2008. New auxin analogs with growth-promoting effects in intact plants reveal a chemical strategy to improve hormone delivery. *Proceedings of the National Academy of Sciences* 105:15190-15195.
Siaut M, Cuiné S, Cagnon C, Fessler B, Nguyen M, Carrier P, Beyly A, Beisson F, Triantaphylidès C, and Li-Beisson Y. 2011. Oil accumulation in the model green alga Chlamydomonas reinhardtii: characterization, variability between common laboratory strains and relationship with starch reserves. *BMC biotechnology* 11:7.
Simon S, Kubeš M, Baster P, Robert S, Dobrev PI, Friml J, Petrášek J, and Zažímalová E. 2013. Defining the selectivity of processes along the auxin response chain: a study using auxin analogues. *New Phytologist* 200:1034-1048.
Spaepen S, Vanderleyden J, and Remans R. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol Rev* 31:425-448. 10.1111/j.1574-6976.2007.00072.x
Steele JH, Thorpe SA, and Turekian KK. 2009. *Elements of physical oceanography: a derivative of the encyclopedia of ocean sciences*: Academic Press.
Sun P-F, Fang W-T, Shin L-Y, Wei J-Y, Fu S-F, and Chou J-Y. 2014. Indole-3-acetic acid-producing yeasts in the phyllosphere of the carnivorous plant Drosera indica L. *PloS one* 9:e114196.
Sun XM, Ren LJ, Zhao QY, Ji XJ, and Huang H. 2018. Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation. *Biotechnol Biofuels* 11:272. 10.1186/s13068-018-1275-9
Tan KWM, and Lee YK. 2016. The dilemma for lipid productivity in green microalgae: importance of substrate provision in improving oil yield without sacrificing growth. *Biotechnology for biofuels* 9:255.
Tilman D. 1994. Competition and biodiversity in spatially structured habitats. *Ecology* 75:2-16.
West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. *Annual review of Ecology and Systematics* 20:249-278.
Wu X, Zhang J, Qin B, Cui G, and Yang Z. 2013. Grazer density-dependent response of induced colony formation of Scenedesmus obliquus to grazing-associated infochemicals. *Biochemical systematics and ecology* 50:286-292.
Yi X, and Dean AM. 2016. Phenotypic plasticity as an adaptation to a functional trade-off. *Elife* 5.

Table 1 Increased resistance of *D. komarekii* cells to various stress conditions after exposure to IAA

	Survival (%)	
	Control	IAA-treated
Heat-shock (40°C, 10 mins)	88.0±5.7	93.4±5.2
Heat-shock (40°C, 15 mins)	74.9±2.5	83.9±4.1*
Heat-shock (40°C, 20 mins)	61.2±19.3	79.5±2.5
Cold-shock (4°C, 24 hrs)	25.2±16.4	50.0±2.6*
Osmotic shock (0.5 M NaCl, 15 mins)	46.4±6.4	48.6±4.9
Osmotic shock (0.5 M NaCl, 30 mins)	34.4±6.9	40.6±6
Oxidative stress (2 mM H_2O_2, 15 mins)	74.7±24.4	79.6±5.7
Oxidative stress (2 mM H_2O_2, 30 mins)	54.5±9.7	68.6±7.3
Acid shock (pH 3.0, 15 mins)	76.8±1.2	87.9±6.7*
Acid shock (pH 3.0, 30 mins)	54.8±20.2	59.4±16.5
Alkaline shock (pH 8.0, 15 mins)	83.7±9.5	79.6±7
Alkaline shock (pH 8.0, 30 mins)	62.6±13.9	77.3±4.9

Reported values are mean of three measurements ± their standard deviations. The significance of differences between groups was determined using Student t tests and analyses of variance. *$p < 0.05$.

Figure legends

Fig. 1 Growth of coenobial algae *Desmodesmus komarekii* in the presence of several auxins, inhibitor of auxin biosynthesis and auxin transport. *D. komarekii* was cultured in the presence of **a** 300, **b** 200, and **c** 100 µM indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene-1-acetic acid (NAA), 2,3,5-triiodobenzoic acid (TIBA), or 4-biphenylboronic acid (BBo). Growth curves of *D. komarekii* for each compound were measured at 1, 3, 5, 7 and 9 days. Error bars represent standard deviation of values for three replicates.

Fig. 2 Mean number of cells per coenobium and proportions of unicells and of two- and four-celled coenobia of *Desmodesmus komarekii* cultured at 300 µM indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations and cells without treatment. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.

Fig. 3 Transmission electron micrographs of *Desmodesmus komarekii* cells under indole-3-acetic acid (IAA) treatment. **a** Through transmission electron microscopy, we confirmed that the morphological changes in coenobia were not caused by cell aggregation but by the vegetative growth of a mother cell. **b** No extracellular matrix was seen on or around the cells, and the connecting strands between cells were highly visible. **c** Dense section of the warty layer can be seen over each coenobial junction (yellow circle). **d** The accumulation of many starch granules (S) and lipid bodies (L) was observed in the large unicells at day 7 after IAA treatment compared with the cells in control groups.

Fig. 4 Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia in three strains of *Desmodesmus armatus* cultured at different indole-3-acetic acid (IAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to the results of a one-way analysis of variance and least significant difference post hoc test. **a**, **b** *D. armatus* JYCA037. **c**, **d** *D. armatus* JYCA041. **e**, **f** *D. armatus* JYCA045.

Fig. 5 Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia in three strains of *Desmodesmus armatus* cultured at different 2,4-dichlorophenoxyacetic acid (2,4-D) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test. **a**, **b** *D*. *armatus* JYCA037. **c**, **d** *D*. *armatus* JYCA041. **e**, **f** *D*. *armatus* JYCA045.

Fig. 6 Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia in three strains of *Desmodesmus armatus* cultured at different naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test. **a**, **b** *D*. *armatus* JYCA037. **c**, **d** *D*. *armatus* JYCA041. **e**, **f** *D*. *armatus* JYCA045.

Fig. 7 Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus armatus* JYCA039 cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.

Fig. 8 Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus communis* JYCA040 cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.

Fig. 9 Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus opoliensis* JYCA043 cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.

Fig. 10 Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus communis* JYCA044 cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.

Fig. 11 Mean number of cells per coenobium and proportions of unicells and two-, and four-celled coenobia of *Desmodesmus intermedius* strain JYCA042 cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.

Figure 1

Growth of coenobial algae *Desmodesmus komarekii* in the presence of several auxins, inhibitor of auxin biosynthesis and auxin transport.

Growth of coenobial algae *Desmodesmus komarekii* in the presence of several auxins, inhibitor of auxin biosynthesis and auxin transport. *D*. *komarekii* was cultured in the presence of **a** 300, **b** 200, and **c** 100 μM indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene-1-acetic acid (NAA), 2,3,5-triiodobenzoic acid (TIBA), or 4-biphenylboronic acid (BBo). Growth curves of *D. komarekii* for each compound were measured at 1, 3, 5, 7 and 9 days. Error bars represent standard deviation of values for three replicates.

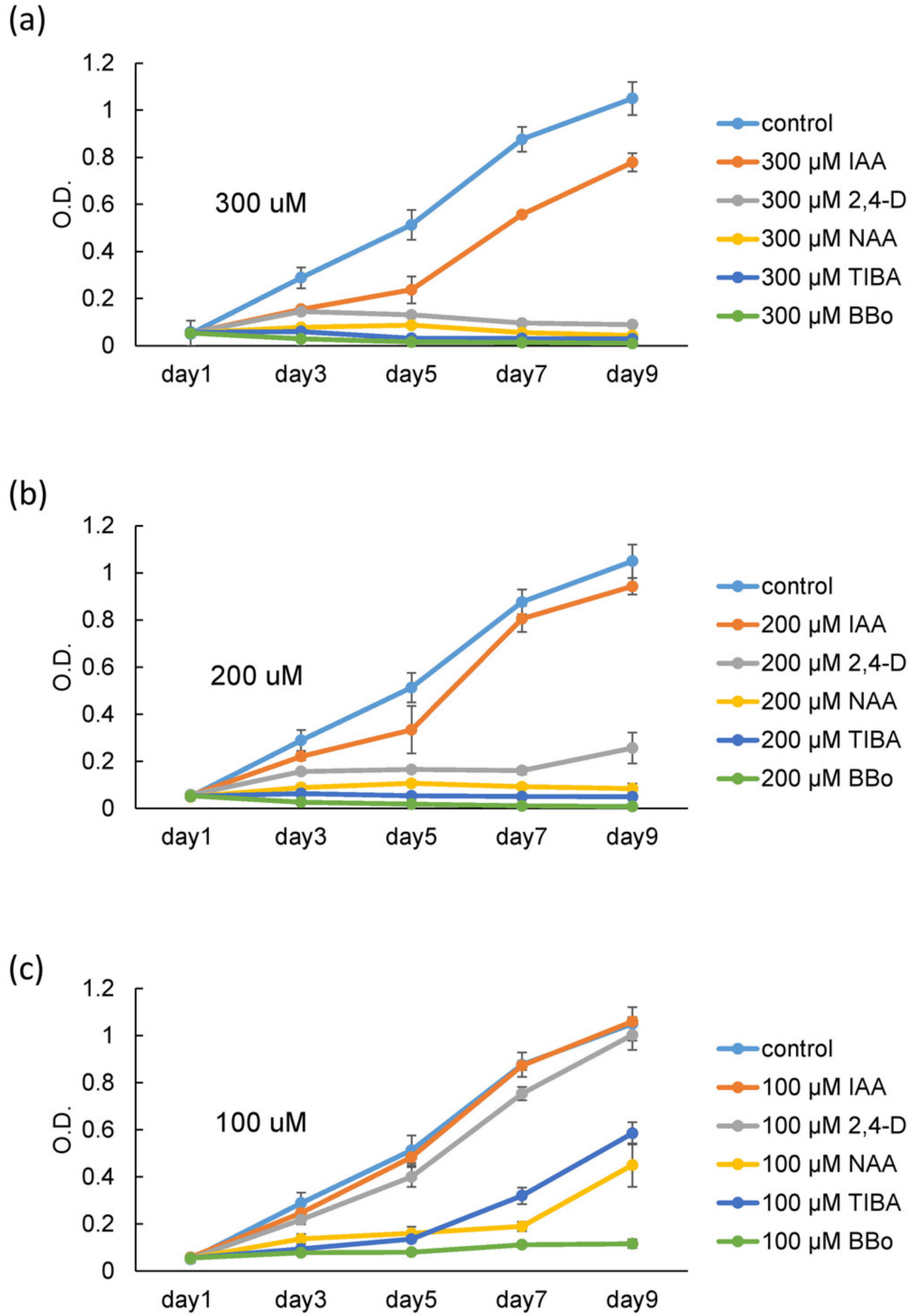

(a)
300 uM
O.D.
1.2
1
0.8
0.6
0.4
0.2
0
day1
day3
day5
day7
day9
control
300 μM IAA
300 μM 2,4-D
300 μM NAA
300 μM TIBA
300 μM BBo
(b)
200 uM
O.D.
1.2
1
0.8
0.6
0.4
0.2
0
day1
day3
day5
day7
day9
control
200 μM IAA
200 μM 2,4-D
200 μM NAA
200 μM TIBA
200 μM BBo
(c)
100 uM
O.D.
1.2
1
0.8
0.6
0.4
0.2
0
day1
day3
day5
day7
day9
control
100 μM IAA
100 μM 2,4-D
100 μM NAA
100 μM TIBA
100 μM BBo

Figure 2

Mean number of cells per coenobium and proportions of unicells and of two- and four-celled coenobia of *Desmodesmus komarekii* cultured at 300 μM IAA, 2,4-D, and NAA concentrations and cells without treatment.

Mean number of cells per coenobium and proportions of unicells and of two- and four-celled coenobia of *Desmodesmus komarekii* cultured at 300 μM indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations and cells without treatment. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.

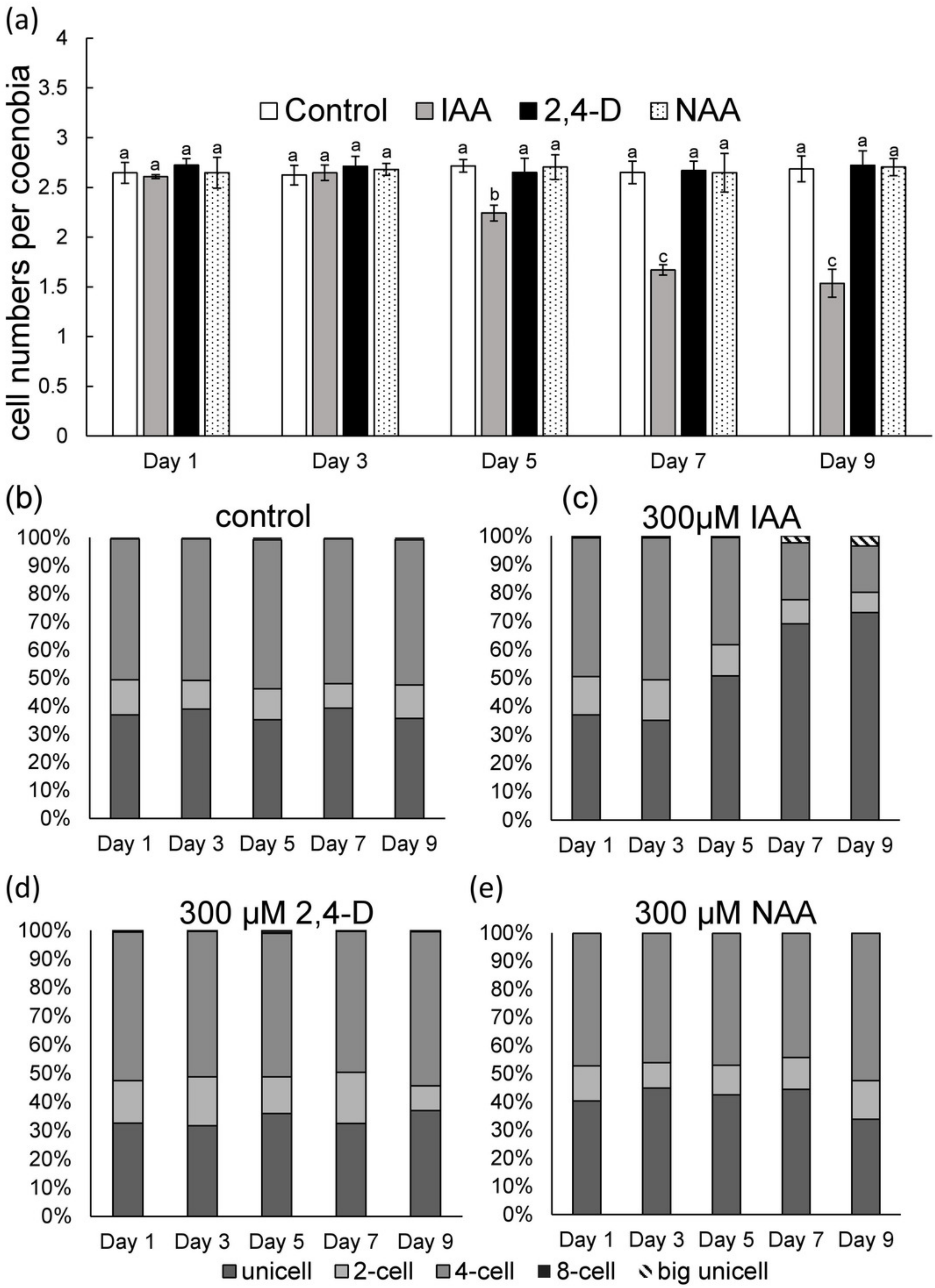

(a)
cell numbers per coenobia
Control
IAA
2,4-D
NAA
Day 1
Day 3
Day 5
Day 7
Day 9
(b)
control
(c)
300μM IAA
(d)
300 μM 2,4-D
(e)
300 μM NAA
unicell
2-cell
4-cell
8-cell
big unicell

Figure 3

Transmission electron micrographs of *Desmodesmus komarekii* cells under indole-3-acetic acid (IAA) treatment.

Transmission electron micrographs of *Desmodesmus komarekii* cells under indole-3-acetic acid (IAA) treatment. **a** Through transmission electron microscopy, we confirmed that the morphological changes in coenobia were not caused by cell aggregation but by the vegetative growth of a mother cell. **b** No extracellular matrix was seen on or around the cells, and the connecting strands between cells were highly visible. **c** Dense section of the warty layer can be seen over each coenobial junction (yellow circle). **d** The accumulation of many starch granules (S) and lipid bodies (L) was observed in the large unicells at day 7 after IAA treatment compared with the cells in control groups.

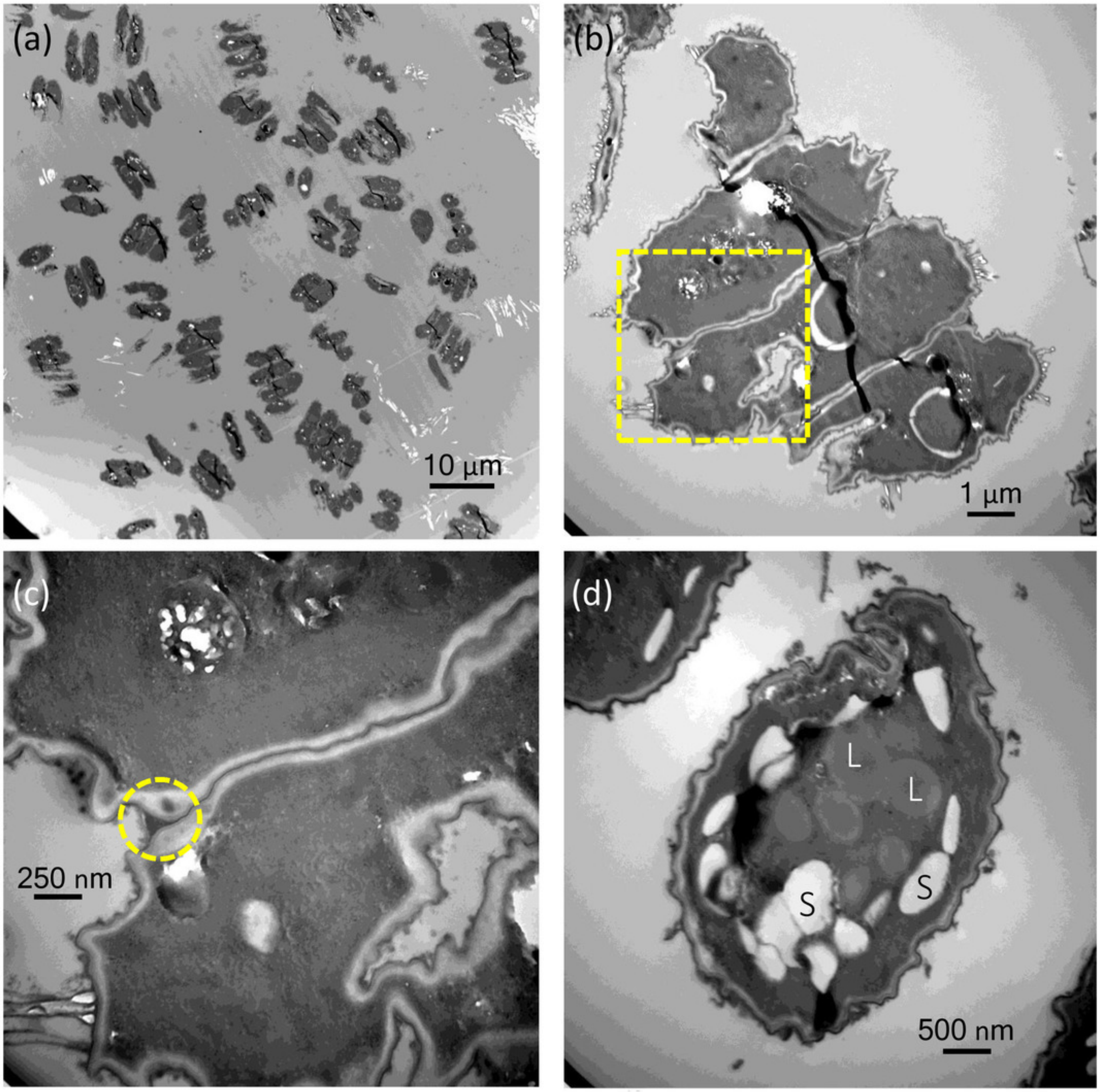

(a)
10 µm
(b)
1 µm
(c)
250 nm
(d)
L
L
S
S
500 nm

Figure 4

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia in three strains of *Desmodesmus armatus* cultured at different IAA concentrations.

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia in three strains of *Desmodesmus armatus* cultured at different indole-3-acetic acid (IAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to the results of a one-way analysis of variance and least significant difference post hoc test. **a**, **b** *D*. *armatus* JYCA037. **c**, **d** *D*. *armatus* JYCA041. **e**, **f** *D*. *armatus* JYCA045.

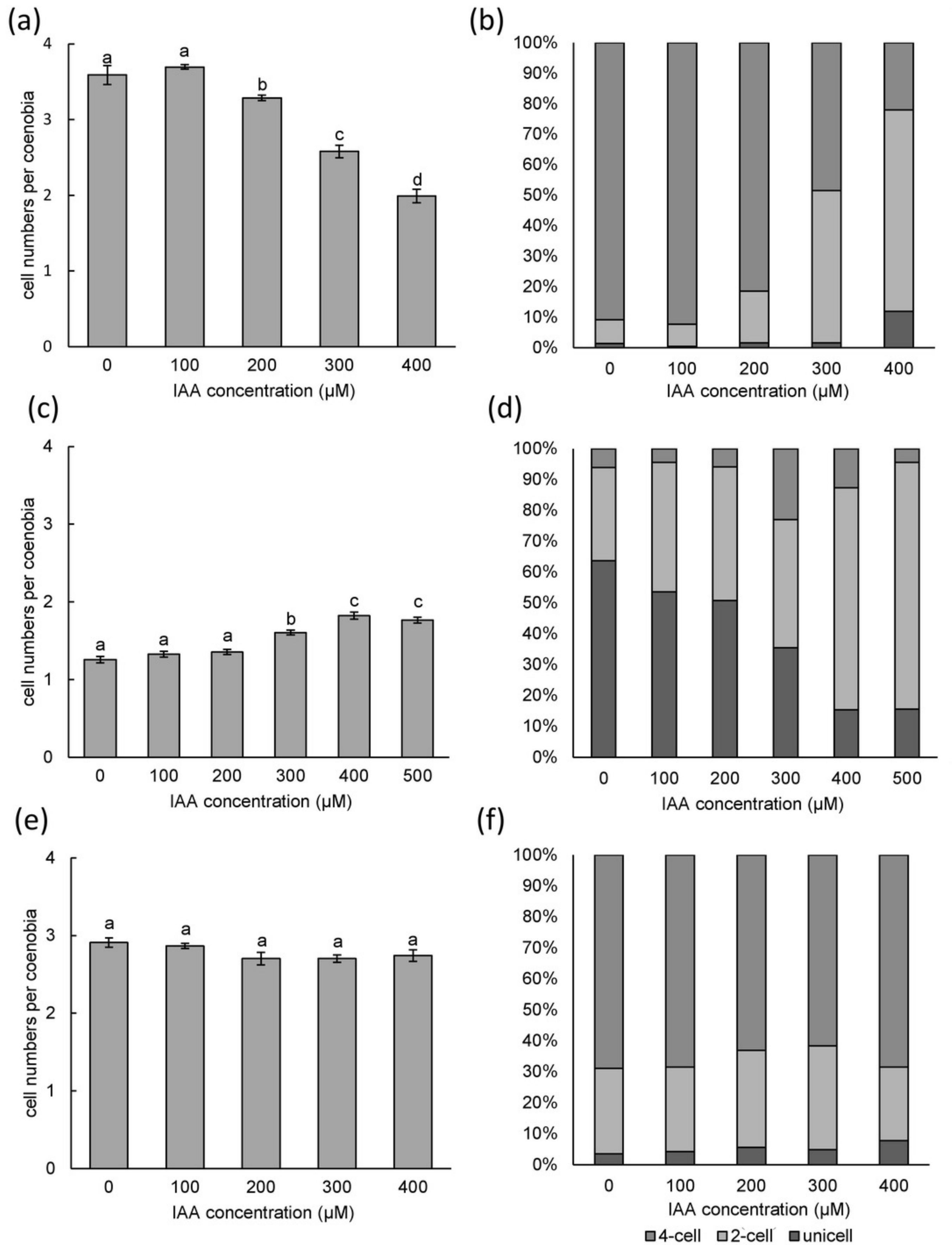

(a)
cell numbers per coenobia
IAA concentration (μM)
(b)
IAA concentration (μM)
(c)
cell numbers per coenobia
IAA concentration (μM)
(d)
IAA concentration (μM)
(e)
cell numbers per coenobia
IAA concentration (μM)
(f)
IAA concentration (μM)
4-cell
2-cell
unicell

Figure 5

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia in three strains of *Desmodesmus armatus* cultured at different 2,4-D concentrations.

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia in three strains of *Desmodesmus armatus* cultured at different 2,4-dichlorophenoxyacetic acid (2,4-D) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test. **a**, **b** *D*. *armatus* JYCA037. **c**, **d** *D*. *armatus* JYCA041. **e**, **f** *D*. *armatus* JYCA045.

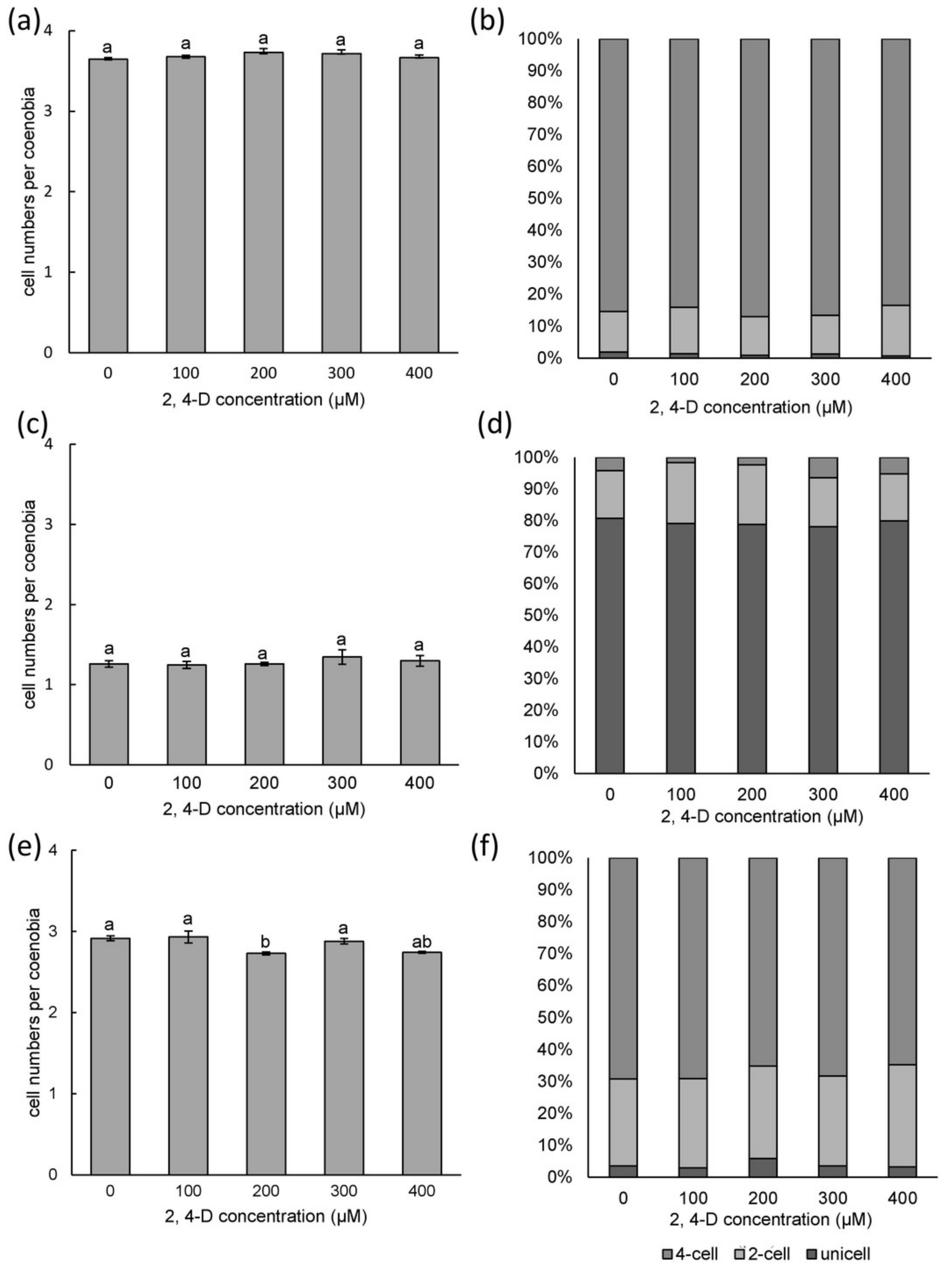

(a)
(b)
(c)
(d)
(e)
(f)
cell numbers per coenobia
2, 4-D concentration (μM)
0
100
200
300
400
a
b
ab
4-cell
2-cell
unicell

Figure 6

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia in three strains of *Desmodesmus armatus* cultured at different NAA concentrations.

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia in three strains of *Desmodesmus armatus* cultured at different naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test. **a**, **b** *D*. *armatus* JYCA037. **c**, **d** *D*. *armatus* JYCA041. **e**, **f** *D*. *armatus* JYCA045.

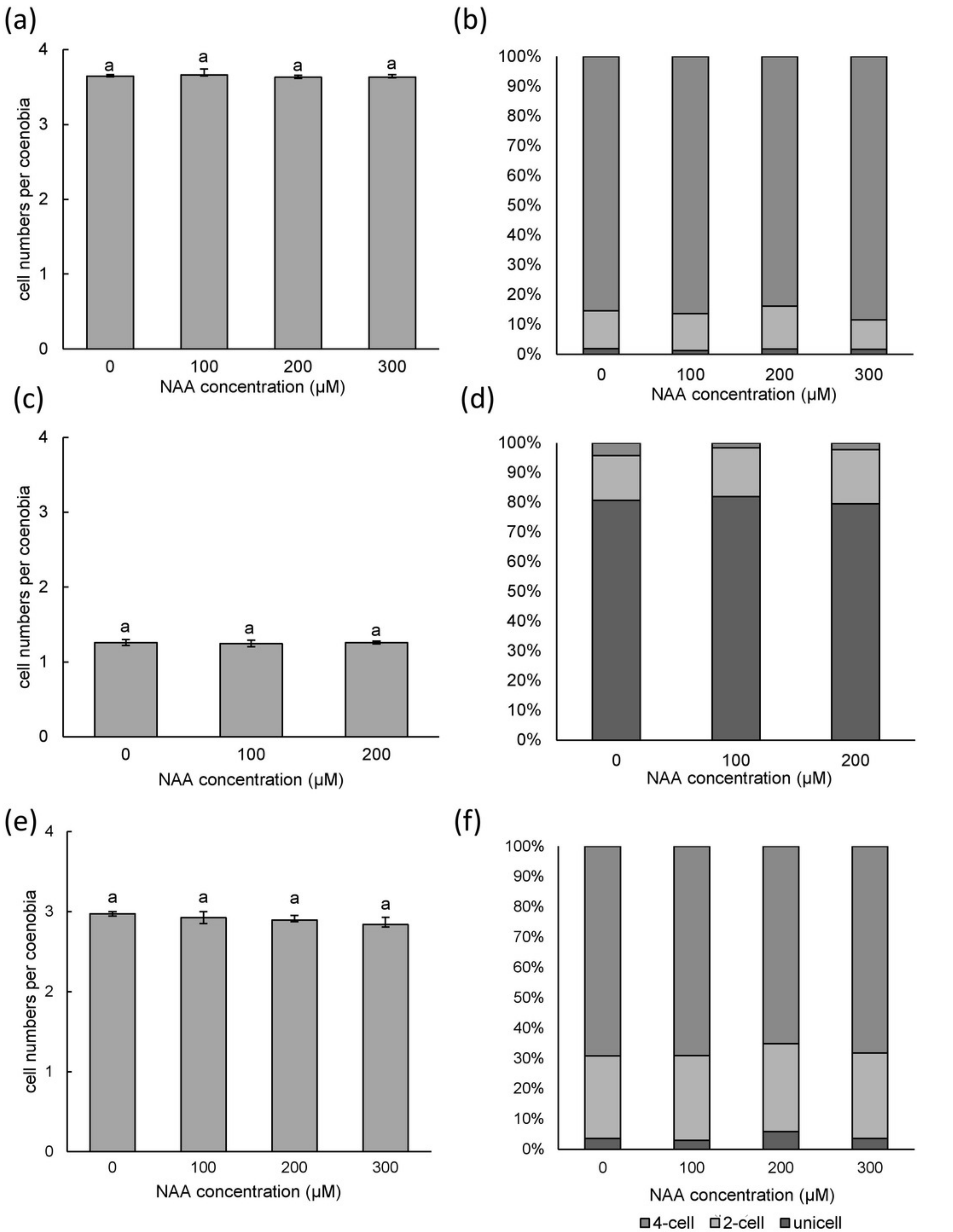

(a)
cell numbers per coenobia
a
0
100
200
300
NAA concentration (μM)
(b)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
NAA concentration (μM)
(c)
(d)
(e)
(f)
4-cell
2-cell
unicell

Figure 7

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus armatus* JYCA039 cultured at different IAA, 2,4-D, and NAA concentrations.

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus armatus* JYCA039 cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.

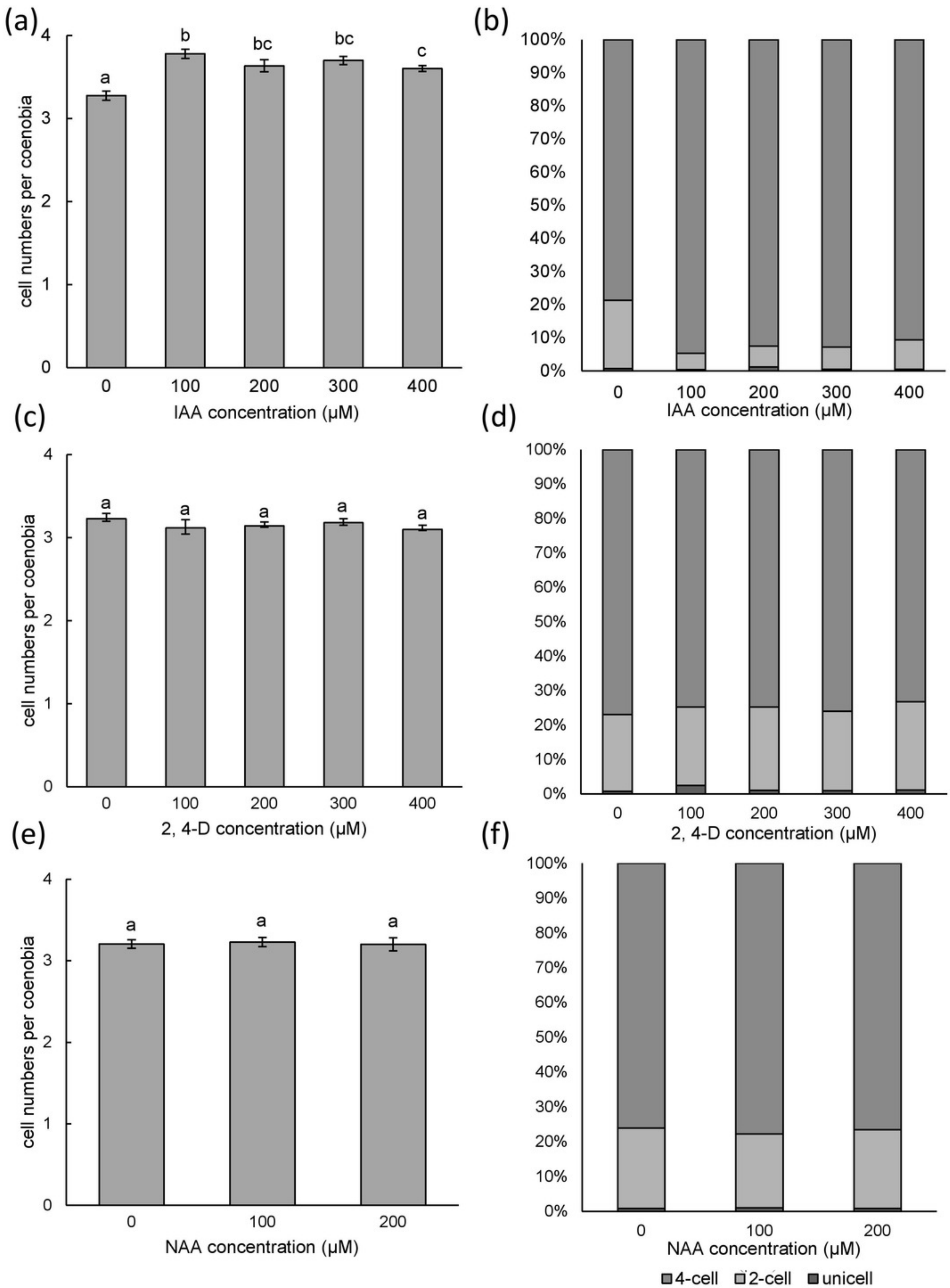

(a)
cell numbers per coenobia
a
b
bc
bc
c
IAA concentration (μM)
(b)
IAA concentration (μM)
(c)
cell numbers per coenobia
2, 4-D concentration (μM)
(d)
2, 4-D concentration (μM)
(e)
cell numbers per coenobia
NAA concentration (μM)
(f)
NAA concentration (μM)
4-cell
2-cell
unicell

Figure 8

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus communis* JYCA040 cultured at different IAA, 2,4-D, and NAA concentrations.

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus communis* JYCA040 cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.

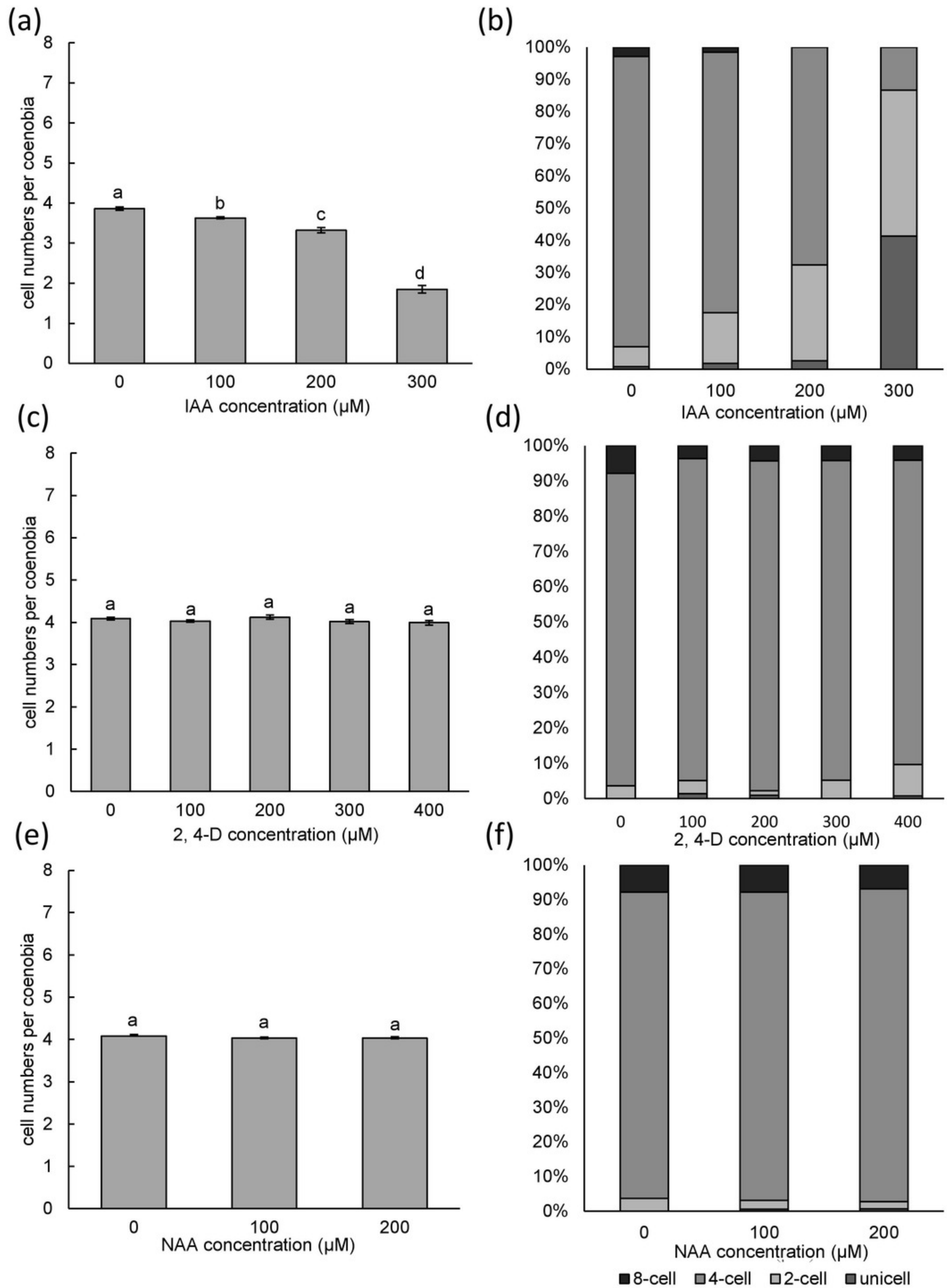

(a)
cell numbers per coenobia
a
b
c
d
IAA concentration (μM)
(b)
IAA concentration (μM)
(c)
cell numbers per coenobia
2, 4-D concentration (μM)
(d)
2, 4-D concentration (μM)
(e)
cell numbers per coenobia
NAA concentration (μM)
(f)
NAA concentration (μM)
8-cell
4-cell
2-cell
unicell

Figure 9

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus opoliensis* JYCA043 cultured at different IAA, 2,4-D, and NAA concentrations.

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus opoliensis* JYCA043 cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.

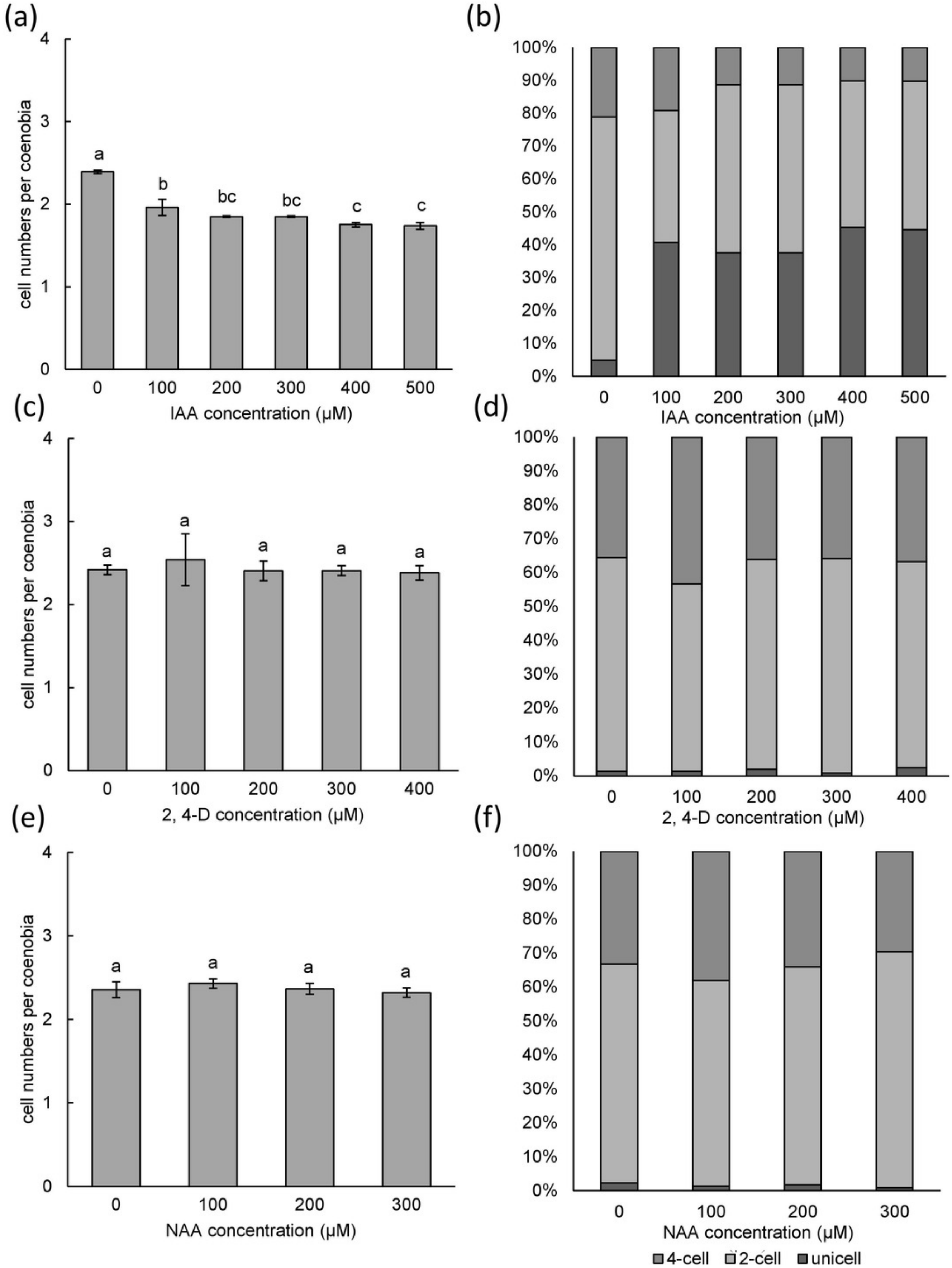

(a)
cell numbers per coenobia
0
1
2
3
4
a
b
bc
bc
c
c
0 100 200 300 400 500
IAA concentration (μM)
(b)
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
0 100 200 300 400 500
IAA concentration (μM)
(c)
cell numbers per coenobia
a a a a a
0 100 200 300 400
2, 4-D concentration (μM)
(d)
0 100 200 300 400
2, 4-D concentration (μM)
(e)
cell numbers per coenobia
a a a a
0 100 200 300
NAA concentration (μM)
(f)
0 100 200 300
NAA concentration (μM)
4-cell
2-cell
unicell

Figure 10

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus communis* JYCA044 cultured at different IAA, 2,4-D, and NAA concentrations.

Mean number of cells per coenobium and proportions of unicells and two- and four-celled coenobia of *Desmodesmus communis* JYCA044 cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.

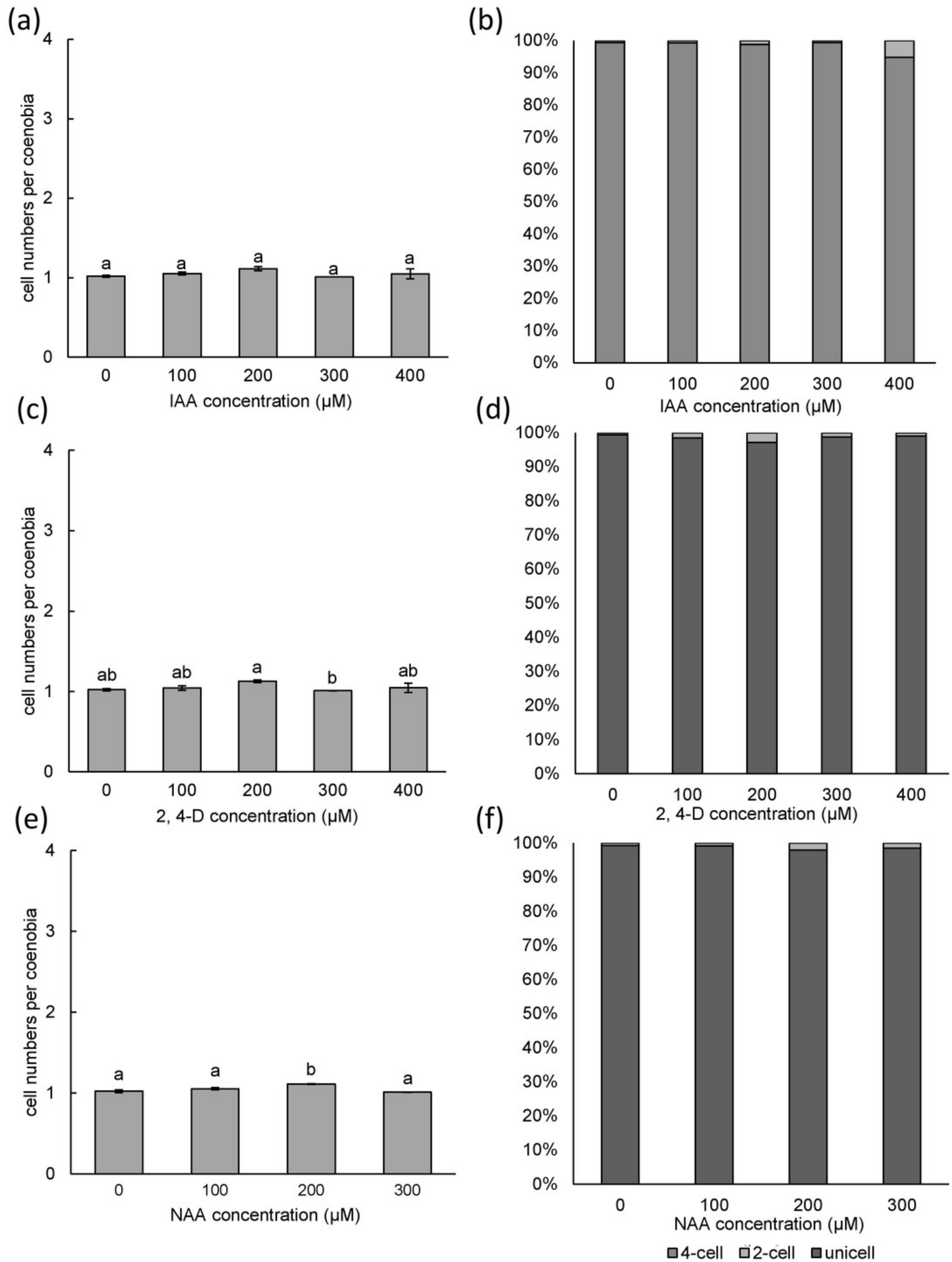
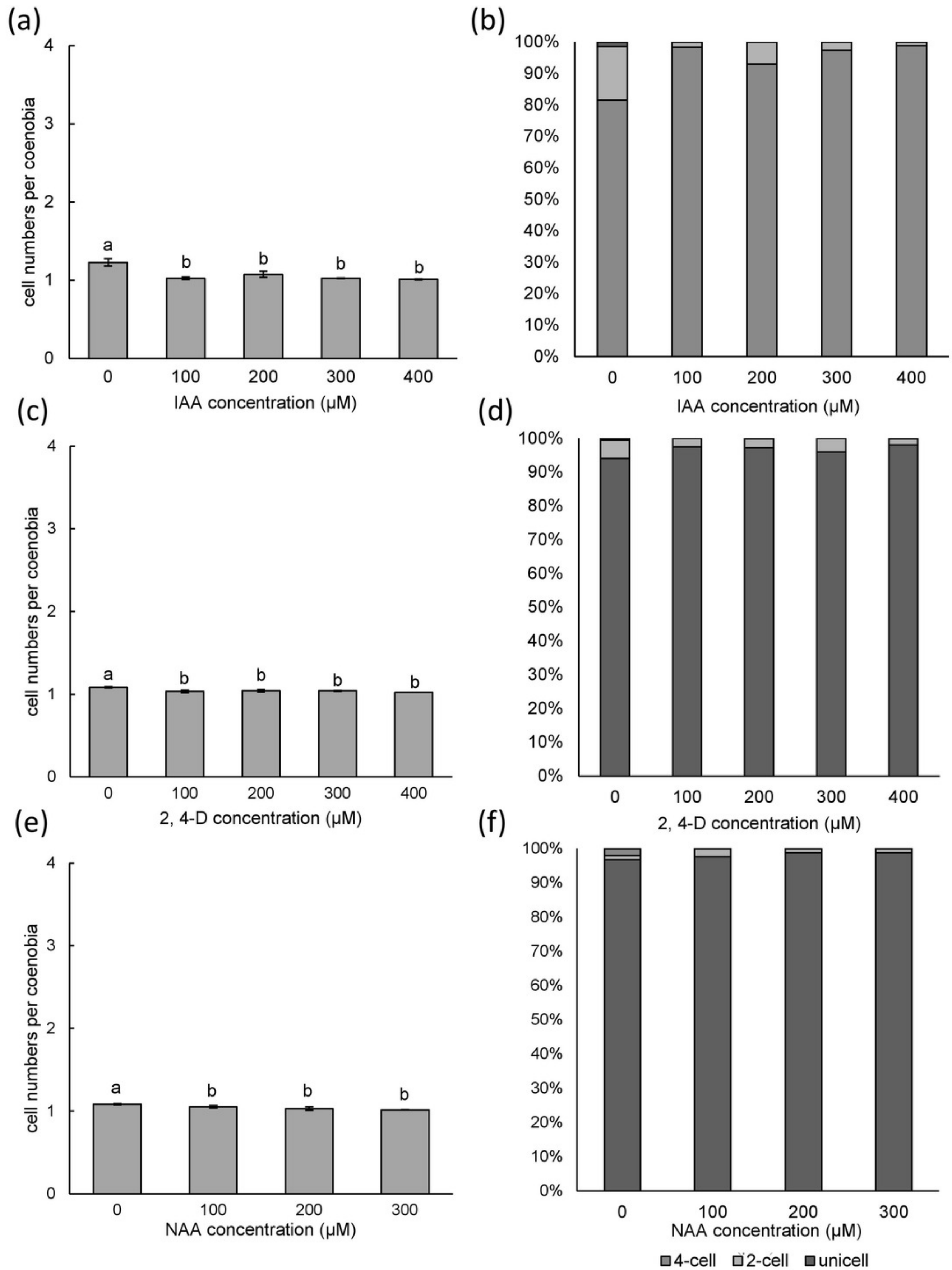

(a)
cell numbers per coenobia
IAA concentration (μM)
(b)
IAA concentration (μM)
(c)
cell numbers per coenobia
2, 4-D concentration (μM)
(d)
2, 4-D concentration (μM)
(e)
cell numbers per coenobia
NAA concentration (μM)
(f)
NAA concentration (μM)
4-cell
2-cell
unicell

Figure 11

Mean number of cells per coenobium and proportions of unicells and two-, and four-celled coenobia of *Desmodesmus intermedius* strain JYCA042 cultured at different IAA, 2,4-D, and NAA concentrations.

Mean number of cells per coenobium and proportions of unicells and two-, and four-celled coenobia of *Desmodesmus intermedius* strain JYCA042 cultured at different indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene-1-acetic acid (NAA) concentrations. Data are presented as means (n = 3) for each group, and morphotype percentages and cell types were based on 200 cell counts in each repeat. Means with the same letter are not significantly different from each other according to a one-way analysis of variance and least significant difference post hoc test.


(a)
cell numbers per coenobia
0
1
2
3
4
a
b
b
b
b
0
100
200
300
400
IAA concentration (μM)
(b)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
100
200
300
400
IAA concentration (μM)
(c)
cell numbers per coenobia
a
b
b
b
b
0
100
200
300
400
2, 4-D concentration (μM)
(d)
0
100
200
300
400
2, 4-D concentration (μM)
(e)
cell numbers per coenobia
a
b
b
b
0
100
200
300
NAA concentration (μM)
(f)
0
100
200
300
NAA concentration (μM)
4-cell
2-cell
unicell