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The Algae Testbed Public-Private Partnership (ATP^3) framework; establishment of a national network of testbed sites to support sustainable algae production

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ABSTRACT

Well-controlled experiments that directly compare seasonal algal productivities across geographically distinct locations have not been reported before. To fill this gap, six cultivation testbed facilities were chosen across the United States to evaluate different climatic zones with respect to algal biomass productivity potential. The geographical locations and climates were as follows: Southwest, desert; Western, coastal; Southeast, inland; Southeast, coastal; Pacific, tropical; and Midwest, greenhouse. The testbed facilities were equipped with identical systems for inoculum production and open pond operation and methods were standardized across all testbeds to ensure accurate measurement of physical and biological variables. The ability of the testbed sites to culture and analyze the same algal species, *Nannochloropsis oceanica* KA32, using identical pond operational and data collection procedures was evaluated during the same seasonal timeframe. This manuscript describes the results of a first-of-its-kind coordinated testbed validation field study while providing critical details on how geographical variations in temperature, light, and weather variables influenced algal productivity, nitrate consumption, and biomass composition. We found distinct differences in growth characteristics due to the geographic location and the resulting climatic and seasonal conditions across the sites, with the highest productivities observed at the desert Southwest and tropical Pacific regions, followed by the Western coastal region. The lowest productivities were observed at the Southeast inland and Midwest greenhouse locations. These differences in productivities among the sites correlated with the differences in pond water temperature and available solar radiation. In addition two sites, the tropical Pacific and Southeast inland experienced unusual events, spontaneous flocculation, and unusually cold and wet (rainfall) conditions respectively, that negatively affected outdoor algal growth. In addition, minor variability in productivity was observed between the different experimental treatments at each site, much smaller compared to differences due to geographic location. Finally, the successful demonstration of the coordinated and standardized operation of the testbed sites established a rigorous basis for future validation of algal strains and operational conditions and protocols across a geographically diverse testbed network.

1. Introduction

Bioenergy from algae has the potential to contribute substantially to the nation's renewable energy future, but significant challenges surround the transition to commercial-scale algae farms [1,2]. Some of the barriers can be attributed to disparate literature reports on productivity and compositional estimates for algae production. The lack of agronomic data on algae cultivation makes future-year projections difficult and unreliable. Increased research and development as well as near- or at-scale demonstrations are needed to improve algal productivity and pond performance, reduce risk and uncertainty in deployment of an algal biofuels process, and address and validate the data gap between assumed and actual experimental values [3,4]. The Algae Testbed Public-Private Partnership (ATP^3) is a consortium that was created to bring together an integrated partnership between academic institutions, commercial enterprises, and National Laboratories with multiple

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testbed field sites in geographically diverse locations to serve as a network of test facilities for the algal biofuels research community (http://atp3.org/about-us/). The objective of the overall project was to determine baseline algal productivity over multiple years of operation through a series of Unified Field Studies (UFS) in order to understand seasonal as well as geographical variability in algal productivity. The UFS were designed to grow the same algal species during the same seasonal time frame across the testbed network. These data are critically important to support techno-economic analysis (TEA) [5], life cycle analysis (LCA) [6–8], resource assessment (RA) (e.g. water, CO_2, and other infrastructure availability) [9–12], predictive growth modeling, and the development of crop protection strategies for the nascent algal biofuels industry. In addition, the publicly available, curated data generated from this consortium serves to support a state of technology assessment for algae bioenergy and bioproducts which could ultimately guide the prioritization of the barriers that need to be overcome prior to commercialization. This need for experimental studies in the context of initiating an agronomic basis for algae productivity projections is present both in the US and globally. The studies that are enabled by the ATP^3 consortium allow for a comparative assessment of productivity and biomass compositional response across diverse geographic regions.

The facilities of the ATP^3 network were originally established to fill a niche requirement of the algae research and industry community to have access to a testbed network where different species of algae could be tested over multiple seasons and locations. The ATP^3 network provides exactly this, along with flexibility in cultivation and processing technologies. The ATP^3 network includes commercial and academic sites with over 1.8 M L of culturing capacity in raceway ponds and closed photobioreactors (PBRs). To support the transition of algae operations to commercial scale, the ATP^3 network has been a major driver in the implementation and support for a uniform language and common methodologies for the characterization of both algae biology as well as biomass compositional characteristics. Throughout this project, the ATP^3 consortium was committed to providing objective standards for data collection, management, quality control, and analysis to ultimately help define and establish standardized metrics for the discussion and dissemination of algal growth metrics and biochemical compositional analyses.

The research portion of the ATP^3 project was designed to investigate the impact of geographical location on algal biomass productivity and composition, similar to theoretical studies that have been described in the literature [13–15]. The ATP^3 testbed sites were selected as they offered geographic and climatic diversity and a spectrum of resources, including natural seawater and/or wastewater, and were equipped with a range of algae production and processing systems with demonstrated cultivation, harvesting, and processing capacity in a variety of configurations (e.g. open/closed ponds/PBRs). The sites represent diverse geographical and climactic locations; Southwest, desert (AzCATI, Arizona State University (ASU), Mesa, AZ); Western, coastal (California Polytechnic State University (CP), San Luis Obispo, CA); Southeast, inland (Georgia Institute of Technology (GT), Atlanta, GA); Pacific, tropical (Cellana LLC (CELL), Kona, HI), Midwest greenhouse (Touchstone Research Laboratory (TRL), Wooster, OH). During the first year of operations, a 6th site was added to the consortium to include a Southeastern coastal region (Florida Algae (FA), Vero Beach, FL) (Fig. 1.A–F).

The influence of physical parameters on algal yield and biomass composition was important to understand in order to draw conclusions and verify RA, LCA, and TEA predictions that have been made and will ultimately guide development and deployment strategies for algal production plants [12,16]. Thus, to minimize variation from non-geographical-related inputs, the algal cultivation system and open pond design used throughout was standardized with respect to size, geometry, volume, depth, and hydrodynamic mixing. Prior to being able to perform the UFS, the testbed network needed to establish the ability to operate in a coordinated manner and thus demonstrate the value of the testbed network. An initial experiment involving growth and data collection at the 5 initial testbed sites was designed (referred to as the validation UFS) to allow pond operators to gain experience with the equipment as well as production and harvesting procedures to ensure that quality data were generated using identical methodology across the testbed sites. Concurrently, identical analytical methodologies were successfully implemented and standardized across the testbed network to ensure that accurate biochemical composition results could be achieved using standard algal biomass reference material. The specific objectives of the validation UFS experiment were: 1) conduct coordinated and controlled algal growth experiments across the testbed network; 2) exercise protocols and identify gaps in procedures, systems, and personnel; 3) collect cultivation data for *Nannochloropsis oceanica* KA32 in terms of growth rate, biomass productivity, and biochemical composition; and 4) collect, manage, and disseminate scientific data to support the algae research community [17]. As such, this communication describes the successful build-out and system validation of the ATP^3 testbed network.

2. Materials and methods

2.1. Outdoor open ponds, inoculation, and pond monitoring

At each of the testbed sites, 6 identical raceway ponds (4.2 m^2 approximate pond surface area with a nominal volume of 1000 L at a depth of 25 cm; Commercial Algae Professionals, http://www.commercialalgae.com) equipped with a YSI 5200A-DC (YSI Inc., Yellow Springs, OH, USA) water quality monitoring system simultaneously measuring pH, pond water temperature (°C), dissolved oxygen saturation (%), salinity (g L^{-1}), and a LiCor LI-190R quantum pyranometer (LiCor, Lincoln, NE, USA) measuring photosynthetically active radiation (PAR; μmol photons m^{-2} s^{-1}). Ponds were also equipped with a stainless steel paddle wheel and a CO_2 sparge line for pH control linked to the YSI online pH probe (Fig. 1.G). Each pond was monitored using a combination of the online equipment described above and manual sampling according to a set schedule (Table 1).

The algae species *N. oceanica* KA32 was originally isolated by Cellana LLC and selected because of robust performance in multiple outdoor cultivation experiments in Hawaii. The alga strain was confirmed to be *N. oceanica* (with 100% homology) through sequencing of genomic DNA across a region of the18S ribosomal subunit using the primers 360FE: 50-CGGAGARGGMGCMTGAGA-30 and 1391RE: 50-GGGCGGTGTGTACAARGRG-30 [18,19].

N. oceanica KA32 was grown in salt water with modified f/2 media, prepared as described with target concentrations of 49 ppm (21 ppm for the low N experiment) NO_3 ($NaNO_3$) and 7 ppm PO_4 (NaH_2PO_4) (ATP^3 Protocol, Modified f/2 Media [17]). Ponds were filled to 24 cm in depth with either seawater or artificial salt water (35 g L^{-1} Oceanic Sea Salt or Instant Ocean) depending on the site and their access to seawater. Culture water in the ponds was sterilized with sodium hypochlorite at 100 ppm chlorine concentration for a minimum of 12 h. After this time period, ponds were checked for the extent of dechlorination using a chlorine test kit. If residual chlorine was detected, the pond water was further dechlorinated with 0.005 mL L^{-1} of 3.2 M sodium thiosulfate for a minimum of 10 min then the pond water was re-checked. Once the pond water was verified to be dechlorinated, N:P stock solutions were added to achieve the above mentioned target nutrient concentrations and the ponds were then ready for inoculation.

In addition to standardizing the outdoor cultivation systems, identical protocols for indoor seed cultivation were also established at each site to allow for uniform production of seed culture in terms of volume and quality (ATP^3 Protocol, Indoor Seed Production in Columns and Panels [17]). Briefly, Cellana LLC shipped 1 L of seed culture to each site. This seed culture was then treated identically at each site as follows: the 1-L volume was split evenly among four 800 mL glass

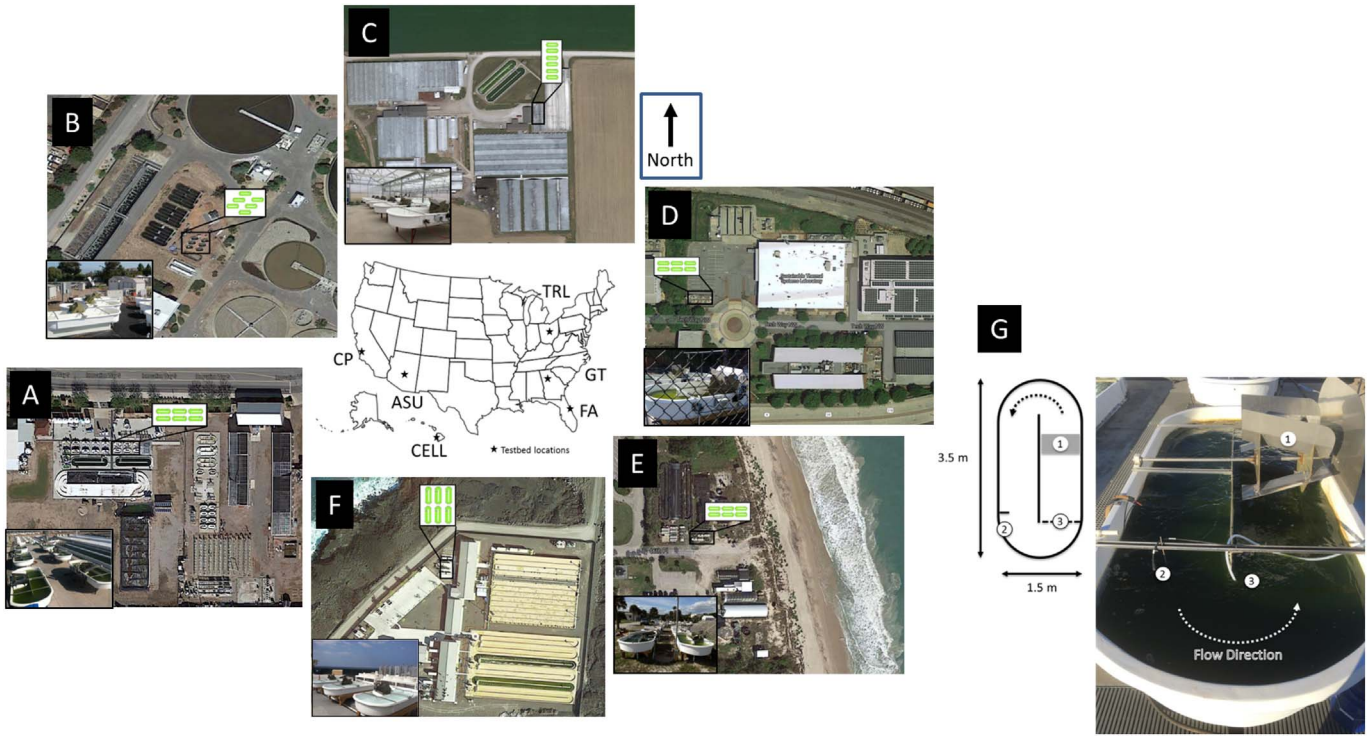

Fig. 1. Geographic location, site overview showing pond orientation, installed pond layout (inset picture), and latitude and longitude of the ATP^3 testbed facilities: (A) ASU: 33.304294, − 111.673536, (B) CP: 35.254055, − 120.674553, (C) TRL: 40.824708, − 81.862825, (D) GT: 33.770844, − 84.403457, (E) FA: 27.675673, − 80.362776, and (F) CELL: 19.734646, − 156.053119, (G) Illustration of typical raceway pond layout as installed across the ATP^3 network; (1) stainless steel paddle wheel, (2) YSI probe cluster for pH, temperature, DO, and salinity measurements, and (3) CO_2 sparge line.

bubble columns (250 mL inoculum and 550 mL fresh media). Columns were run under continuous cool white fluorescent light (~150 μmol m^{-2} s^{-1}) with continuous bubbling with 2% CO_2/air (v/v). Once a culture density of approximately 0.8 g L^{-1} ash free dry weight (AFDW) was reached, the four columns were then split four ways to a total of 16 columns. Once the column seed cultures reached a density of approximately 2.5 g L^{-1} AFDW, 14 of the columns were used to inoculate fourteen 2′ × 2′ vertical flat panel reactors each with a 2″ light path and a nominal 15 L volume. Two of the columns were retained as backup seed culture in the event they were needed for pond reset due to a failure. The same lighting and 2% CO_2/air v/v was used as for the columns. The panels were started at an initial target density of approximately 0.15 g L^{-1} AFDW. Once the panel cultures reached a minimum density of 1.5 g L^{-1}, they were then combined into a single master batch inoculum and used to inoculate six outdoor ponds at a minimum target density of 0.05 g L^{-1} AFDW. Typical duration for seed culture scale-up from columns through to panels and finally to outdoor ponds was 18–21 days.

2.2. Culture and productivity monitoring

During the outdoor cultivation experiment the following measurements were carried out according to Table 1. Dry weight (DW) and AFDW were measured using a standardized method (ATP^3 Laboratory Analytical Procedure (LAP); Gravimetric Method for Determination of Dry Weight (DW) and AFDW [17,20]). Briefly, glass microfiber filter papers (VWR #28333-139) were pre-ashed overnight in a glass Petri dish at 500 °C and thereafter stored under vacuum desiccation. Pre-ashed filters were weighed in foil weighing pans on a 4-place balance. Filters were then placed on a filtration apparatus and covered with a magnetic or glass funnel. Filters were pre-wetted with distilled water and well-mixed culture was filtered. The filter was then rinsed 3 times with ammonium formate (0.5 M) including the edges after removal of the funnel. The filter was then placed back in its weighing tin and placed in a 105 °C drying oven overnight. Filters were then removed to a desiccator for cooling to room temperature and weighed to give DW. The filters were then ashed again at 500 °C for 4 h, removed to vacuum desiccator to cool, and finally weighed to give AFDW. At ASU and CELL,

Table 1
Sample data, units, schedule, and method for data collection.

Sample	Units	Schedule	Sample method
OD_{750}		Sunrise (+ 30 min) M–F	Manual
DW, AFDW	g L^{-1}	Sunrise (+ 30 min) M, W, F	Manual
Composition (ash, lipid, carbohydrate, protein)	% AFDW	Sunrise (+ 30 min) weekly or as change in conditions warrants	Manual
Nutrients (nitrate, phosphate)	mg L^{-1}	Sunrise (+ 30 min) M, W, F	Manual
Weather data (air temperature, % relative humidity, global light energy, precipitation, wind speed and direction)	°C, % RH, W m^{-2}, cm, km h^{-1}, degrees	Hourly	Internet weather sites
In-situ sensors (pH, pond water temperature, salinity, % oxygen saturation, PAR)	°C, g L^{-1}, %, μmol photons m^{-2} s^{-1}	15 min sampling intervals	YSI5200
Manual pond checks (pH, pond water temperature, depth with paddlewheel off)	°C, cm	M–F; AM and PM	Manual
Microscopic check for contaminating organisms	Brightfield, 40 ×–100 × magnification	Weekly and at final harvest	Manual

nitrogen and phosphorous were measured using set protocols available for a Lachat autoanalyzer, Quickchem 8500 with flow injection analysis. In brief, nitrate was quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) was then determined by diazotizing with sulfanilamide followed by coupling with *N*-(1-naphthyl)-ethylenediamine dihydrochloride. The absorbance of the resulting water soluble dye was measured at 520 nm. At CP, FA, and GT, nitrogen was measured using a HACH test kit, based on a modification of the Cadmium Reduction method using gentisic acid in place of 1-naphthylamine and uses a sensitive chromotropic acid indicator (Model NI-12, Hach, Loveland, CO).

To monitor contamination in the ponds, a protocol using a microscope was implemented across all the sites. Routine contamination identification was carried out at a minimum of 40 × magnification for three samples per pond periodically throughout the experiments (minimum 1 ×/week and at any harvest event). Major categories of contamination (e.g., rotifer, ciliate, amoeba, flagellated, and other algae) were tracked.

For a subset of the data points, biochemical composition data was collected. The methods for compositional analysis were as described before, with minor modifications to allow for the implementation at individual testbed sites [21–26]. These data and alignment metrics are described in an accompanying manuscript [27].

2.3. Continuous environmental data collection

For each site, weather data were collected either on site (ASU and CELL) or using publicly available data in close proximity (within 6 miles) to the site. For CP, data from the California Irrigation Management Information System, San Luis Obispo station #52 were used and for the GT site, data from the Clark Atlanta University weather station were used [28]. For the FA site, data were from Weather Underground, station: KFLVEROB15. For the TRL site, data were from the National Solar Radiation Database delineated by the latitude and longitude of the TRL site [29]. Data collected included air temperature (°C), relative humidity (RH, %), global light energy ($W\,m^{-2}$), wind speed ($km\,h^{-1}$), and wind direction (deg).

2.4. Experimental replication, quality control, and statistical treatment

Each site was responsible for data collection and aggregation into standardized spreadsheets. Data consisted of manual and online pond measurements, laboratory characterization of pond samples, and data from weather stations according to the sampling schedule (Table 1). A standardization strategy as quality control on replicate measurements was implemented across individual sites to achieve a targeted 10% relative inter-site variability for these measurements. Collected data was analyzed for statistical variability using JMP 13 Statistical Software (SAS Institute, Cary, NC). Measurement precision data was compared based on interquartile ranges of the collected measurements as well as the 95% confidence interval. Precision metrics used were relative standard deviation (RSD, also referred to as coefficient of variation, CV). The complete validation UFS dataset is publically available on the Open Energy Information (OpenEI) website (http://en.openei.org/wiki/ATP3) as UFS-1 Experiment (Oct–Dec 2013).

3. Results and discussion

A prerequisite for comparing data across sites was an understanding of the uncertainty around data collected at each site. In order to reduce experimental uncertainty due to using different protocols and personnel, prior to the validation UFS experiment, each of the sites standardized the protocols and procedures that underpin data collected for nutrient and productivity measurements. The goal of this standardization was to train personnel, exercise the protocols, and set a baseline for the variability that was inherent in an experimental framework involving data collection across 6 different testbed sites. Of the 6 sites, 5 participated in the validation UFS while the Florida Algae site was only fully operational after the validation UFS experiment. However, the alignment of protocols and procedures were carried out across all 6 sites and the data are presented together.

Throughout the experimental results presented here, the foundation of statements regarding biomass accumulation and productivity observed between treatments and across sites is set in the accurate measurement of AFDW accumulated for each pond at regular intervals. Because AFDW is a crucial measurement to the entire framework we establish here, we have dedicated a significant effort to test the inter-site variability of AFDW measurements. A second major variable that relies on highly accurate measurements is nitrogen consumption by the algal cultures, and thus each site performed an additional validation of their quantitative nitrogen measurements. To achieve this initial validation, a total of five reference samples with 2 (15 and 30 ppm N as nitrate ($mg\,L^{-1}$)) and 3 (0.05, 0.52 and 0.92 $g\,L^{-1}$ AFDW *N. oceanica* KA32 cell suspension) concentrations of nitrogen and AFDW respectively, were distributed to each of the sites from a single location (ASU). Each of the sites measured each sample as 5 replicate measurements and the data were collected and compiled. These data are shown in Fig. 2 and illustrate the intra- and inter-site variability of the measurements.

For both nitrogen and AFDW there were biases observed among the sites, e.g. CP and GT consistently measured nitrogen higher than the prepared concentration. The basis of the high bias of the nitrogen measurements for CP and GT can be found in different underlying methodology used for nitrate measurement; CP used a Kjeldahl method [30], whereas GT used an ion chromatographic method, relative to the other labs using a spectrophotometric kit as described in the Materials and methods section. For the AFDW measurements, CELL and FA measured a higher than average AFDW concentration in the provided reference samples. The data in aggregate correspond to a coefficient of variation or relative standard deviation of 13–15% for nitrogen concentration and 7.9–10.6% for AFDW. Thus, the differences in the absolute measurements of these variables between the sites have to be larger than the inter-site variability quantified by this initial alignment study to be statistically significant. For example, a difference of less than 15% in the measured nitrogen concentration at two different sites is therefore not statistically significant.

In a similar manner for alignment purposes, the automated measurement probes were tested at each site to ensure proper functioning both for probe variance when in the same pond and probe variance when measuring 3 different ponds filled with identical media. The probe alignment data at the ASU site are shown in Fig. 3. The data illustrates that after calibration, all probes measured identical pH and temperature profiles when submerged in one pond (Fig. 3.A) and when submerged in each of 3 ponds during operation (Fig. 3.B) over a period of 5 days. The variability observed in the absolute measurements of dissolved oxygen between the ponds was likely due to photosynthetic activity and pond physiological parameters. The oxygen saturation data shown indicate that these data can be useful for relative comparisons and interpretation within a pond, but may not be useful for inter-pond or inter-site comparisons due to pond-specific biases in the DO probe data.

Once the seed trains, outdoor ponds, monitoring equipment, and analytical methods were established and standardized at each testbed site, the initial growth validation experiment was implemented across the testbed network to demonstrate coordinated pond operation using the same algal species and the same experimental protocols (Fig. 4).

The seed train was inoculated and approximately 21 days later, the outdoor ponds were inoculated. The validation UFS experiment was designed to examine two variables, pH and initial nitrogen content, in triplicate during two experimental periods of approximately 4 weeks each for a total duration of 8 weeks of open pond cultivation. After the

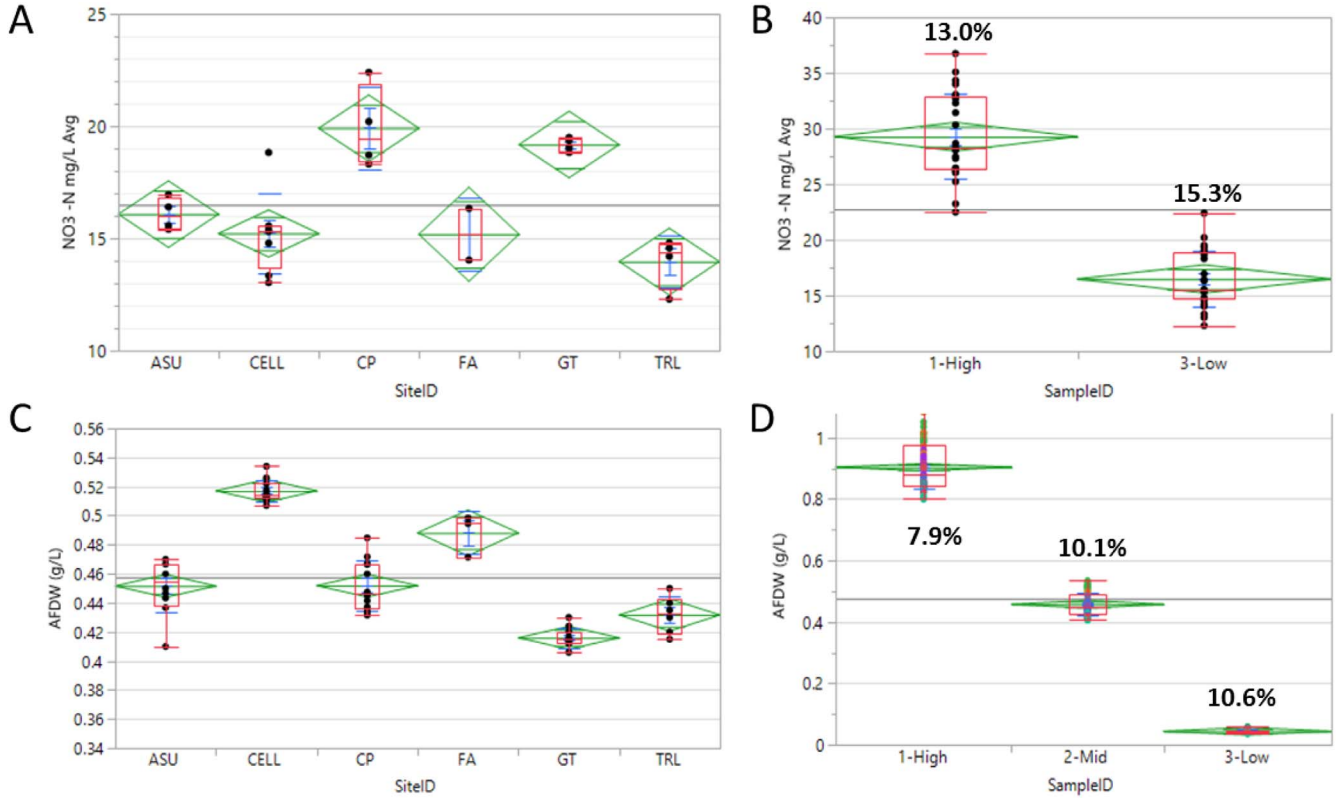

Fig. 2. Overview of the standardization of nitrogen and AFDW measurements showing intra- and inter-site precision. (A) Box-and-whisker plot of measurements on a nitrogen reference sample (15 mg L^{-1}) analyzed at each of 6 testbed sites. Black points represent individual measurements with the horizontal grey line showing the overall mean. The red box encompasses the range from 25% quantile to 75% quantile and the whiskers extend to 1.5 × the interquartile range. Green diamonds show the individual group mean in the center with 95% confidence interval (top to bottom of diamond) with internal horizontal lines showing overlap between the groups. The blue horizontal lines show the standard deviation and the blue vertical line is the mean error bar for the respective data set. (B) % RSD illustrating inter-site precision for each of two reference nitrogen concentrations (30 (high) and 15 (low) mg L^{-1}). (C) Box-and-whisker plot of AFDW measurements on a reference sample (0.5 g L^{-1}). (D) % RSD illustrating inter-site precision for each of three AFDW concentrations (0.05, 0.52, and 0.92 g L^{-1}) of the reference samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

first 4 week pH experiment, the ponds were drained leaving 10% of the volume as inoculum and refilled with medium having two different initial nitrogen concentrations. During these coordinated experiments, the ponds at each site across the testbed network were subjected to actual outdoor conditions (e.g. rain, temperature fluctuations, objects blowing into ponds, contamination, equipment and calibration issues, etc.) particular to each site to examine the effects of the climatic conditions of each geographic local overlaid on the impacts of pH or initial media nitrogen concentration. With these two coordinated experiments, the sites were able to exercise the entire system from seed production to inoculation of replicate ponds, calibration and maintenance of the automated sensors, sampling protocols, and compositional analysis procedures.

For the duration of the validation UFS experiment, starting the 2nd week of October through the 1st week of December 2013, the environmental parameters PAR, pond and air temperature, light energy, and relative humidity were monitored for each of the sites and a subset of 8 days in the middle of the experiment is shown in Fig. 5.

Pond water and air temperatures as well as PAR were declining through the course of this experiment because of the transition into the winter season. Considerable variability in light availability between each of the sites existed with the lowest daily intensity at the Midwest greenhouse location (TRL) and the highest intensity at the Southwest desert location (ASU), which was closely matched by the Tropical Pacific location (CELL). Similarly, temperature fluctuations between the sites over the same 8-day interval illustrated differences in geographical location and a clear correlation between air and pond temperature measurements. The water temperature of ponds located in the temperature-controlled greenhouse at TRL fluctuated less than 5 °C whereas pond temperature fluctuations at all of the other sites could exceed 10 °C over the day-night cycle.

Optical density measurements at 750 nm (OD_{750}) were used as a rapid estimate of biomass accumulation in the ponds, but the direct relationship with actual biomass accumulation (as AFDW) may not be considered absolute. For each of the sites, we compiled data collected across the entire validation UFS experiment to study the OD_{750} to AFDW correlation. For each of the sites, the correlation was found to be linear, but the slope of the correlation was significantly different between each of the sites (Fig. 6).

The dramatic differences observed for the correlation between AFDW and OD_{750} between the sites are, in our opinion, primarily driven by variability in spectrophotometer instruments available at each of the sites. We confirmed the trends of distinct responses and the impact on absorbance measurements early on during our alignment and validation work (Fig. 2 and additional data not shown). However, some of the discrepancies within sites could also be driven by the biology of the cells as influenced by the different climatic parameters the culture was experiencing such as nutrient depletion that was induced in a subset of the ponds during the second experiment (Fig. 6). Additional variation in cell size or number, chlorophyll or other pigment content, cell clumping, and contaminating bacterial or other algae load is also likely to impact absorbance measurements. Anomalies can also be observed in these correlations, such as for example an unexplained spontaneous flocculation event in one of the ponds at CELL caused the correlations of the OD_{750} measurements to differ and, due to the flocculation event, the experiment was terminated early (inset in Fig. 6). Furthermore, the large variation observed at GT for the OD_{750}

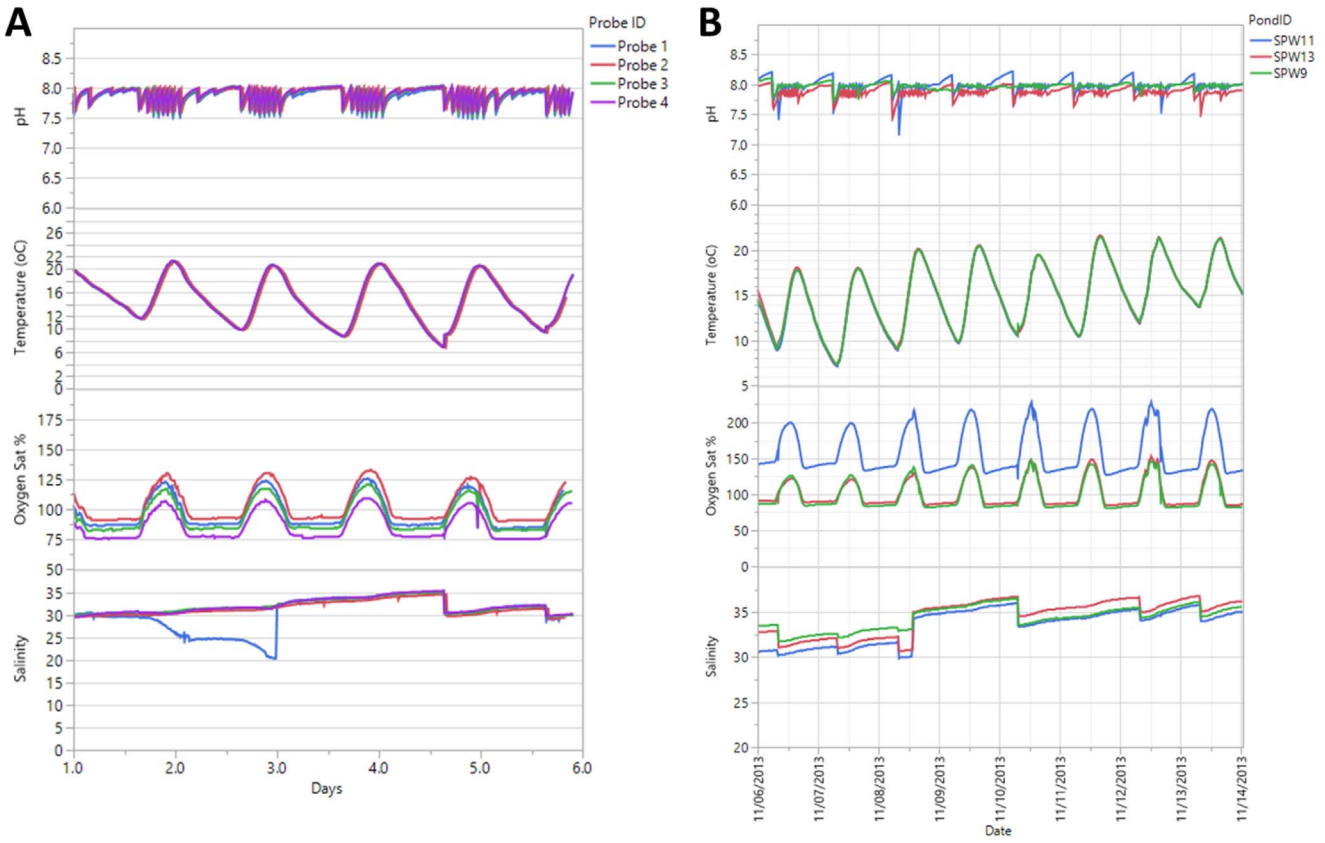

Fig. 3. Continuous monitoring probe alignment test. Each of four sets of probes measured pH, pond temperature (°C), dissolved oxygen saturation (%), and salinity (g L^{-1}). (A) All four probes were set in the same pond for 5 days and (B) a portion of the data showing probe alignment from three different ponds during the course of the validation UFS.

to AFDW correlation could be caused by debris, in particular leaves from nearby trees that blew into the ponds, and to unseasonably cold, rainy, and windy fall weather. This variation was reduced at the mid-way point as the weather improved (i.e., less wind) and trees no longer had leaves. Thus, correlations could be used within a particular site or spectrophotometer to calculate an estimated AFDW at given OD_{750} but could not be extrapolated across the testbed network.

For the first experiment of the validation UFS, pH was set at 7.8 or 8.5 to determine the effects of pH on biomass productivity and biochemical composition of *N. oceanica* KA32. Biomass accumulation (OD_{750} and AFDW), nitrogen (mg L^{-1}), pond depth (cm, accounting for rain events), total biomass accumulation per pond (g, corrected for rain events), and calculated daily productivity (g m^{-2} day^{-1}) for the complete validation UFS experiment are presented in Fig. 7.

During the first three-week pH experiment, no clear differences in AFDW accumulation were noted between the two pH levels (Fig. 7.A). The increase of OD_{750} and biomass accumulation (AFDW) were distinct among the sites, with the highest rates of accumulation observed for the ASU and CP sites and the slowest rates for GT and CELL. The variability between the replicate treatments was low for most of the sites (quantified as relative standard deviation of replicate pond measurements, Fig. 7.B), with the exception of data collected at GT for nitrogen concentration and AFDW. The inter-site variability observed reflects the complexity of setting up a new set of experiments and supporting analyses at different sites. During subsequent experiments at GT the variability was greatly reduced as reflected in the second experiment during the validation UFS.

For the follow-on initial nitrogen concentration experiment, 90% of the culture was harvested from the ponds leaving 10% as an inoculum. The ponds were refilled with media having a high or low (49 vs. 21 mg L^{-1}) initial nitrogen concentration (as $NaNO_3$) to allow for complete nitrogen exhaustion in the case of the lower concentration. The nitrogen starvation period was extended beyond the original planned 3 weeks to observe lipid accumulation over an extended

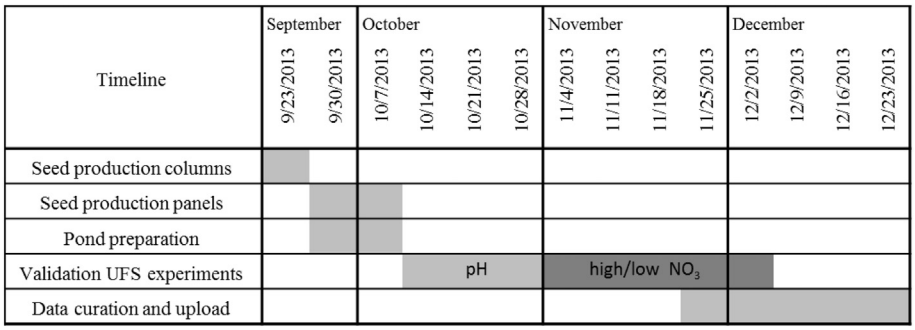

Fig. 4. Planned timeline of the validation UFS experiment. Shading indicates a week of seed column grow out followed by two weeks of seed panel grow out with concurrent pond preparation. After seed grow out, a 3-week pH experiment and a 5-week high vs. low initial nitrogen experiment were planned followed by data curation and upload.

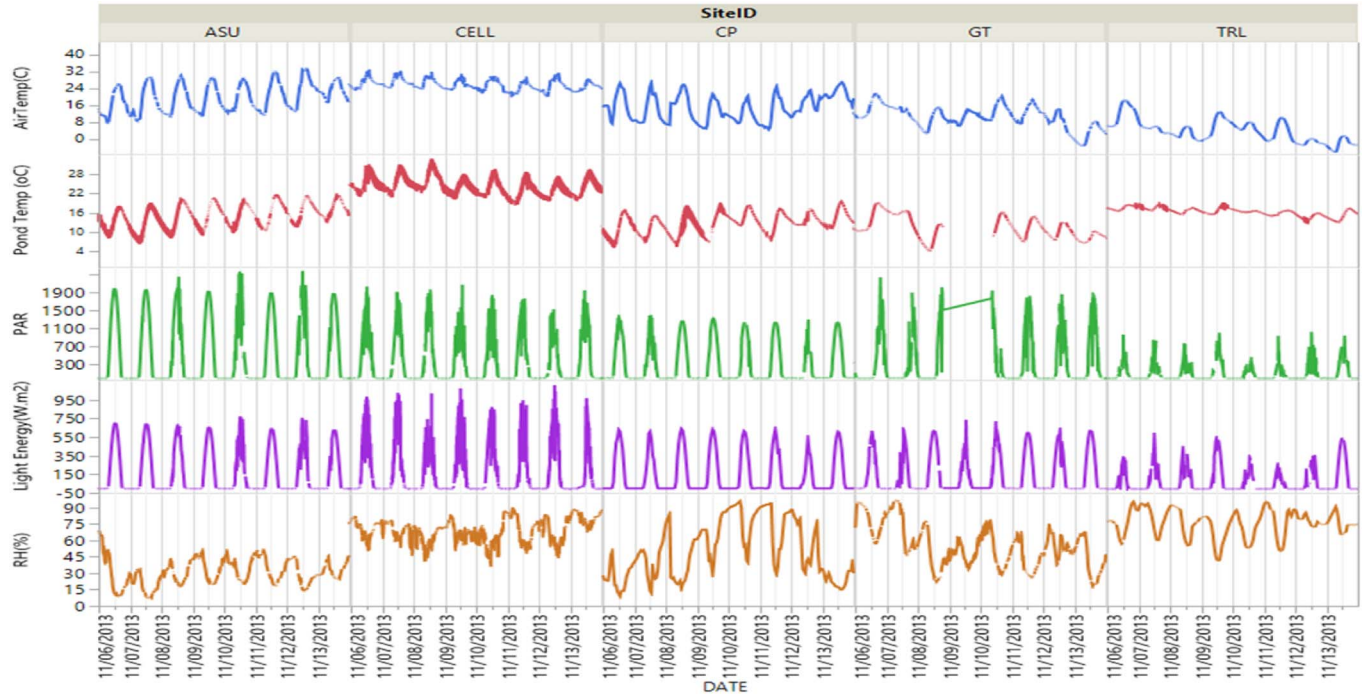

Fig. 5. Continuously measured weather parameters and pond water temperature at each of the sites during an 8 day interval (November 6–November 14, 2013) of the validation UFS experiment; (top to bottom) air temperature (°C), pond temperature (°C), PAR (μmol photon $m^{-2} s^{-1}$), light energy ($W m^{-2}$), and relative humidity (RH, %).

amount of time once the initial nitrogen was exhausted from the culture. For each of the sites, a dramatic reduction in the nitrogen concentration was observed across the two treatments, with the ponds having a low initial nitrogen concentration becoming depleted after the first 10 days. Along with this depletion of a primary nutrient source, we observed a distinct growth rate differential between the treatments only at ASU and CP with the low initial nitrogen ponds exhibiting a decrease in biomass accumulation growth during the last week of the experiment as reflected in the AFDW, OD_{750}, and total biomass accumulation (Fig. 7.A). A larger distinction was observed in the OD_{750} data, where the different treatments showed a ~10% reduction in optical density. This difference could be due to changes in absorbance characteristics of the culture once the nitrogen is consumed and the cultures became nutrient stressed in the low initial nitrogen concentration ponds as discussed above and shown in Fig. 6. This observation underscored the need to collect data in the form of AFDW in order to accurately reflect the cell biomass accumulation in the ponds during different treatments, whereas the OD_{750} data could be used, but may underestimate the total biomass yields reported. This decrease biomass accumulation was not noted at GT or TRL in the nitrate deplete ponds. At CELL it appeared that biomass accumulation was just beginning to slow down as nitrate had been gone for at least a week while the nitrate was continuing to be consumed in the high nitrate ponds. It has to be reiterated that the experiment at CELL was terminated early due to a spontaneous flocculation event. Had this not happened, a difference in biomass accumulation due to nitrogen stress would likely have been observed. Similarly at GT, nitrogen in the high N ponds continued to be consumed but the abnormally cold and rainy winter likely did not provide sufficient environmental conditions for a difference to be clear. Furthermore, biomass accumulation at GT for the second three-week experiment was much lower than at the other sites, again reflecting the low temperature and light availability present in the southeast during mid-winter (on some occasions, the pond water temperature dropped to 0 °C), however had the experiment been continued another week, a difference in biomass accumulation may have been noted. At TRL, there was no difference in initial N due to a mistake in media preparation, where both ponds for the N experiment were inoculated with 100 ppm nitrate instead of the 50 and 20 ppm respectively for the high and low N ponds. Thus growth was not expected to be different between the different sets of ponds. This is confirmed by identical growth rates measured as daily productivities.

Daily productivity was derived from the daily change in AFDW and was calculated for each sample point where an AFDW was recorded. Daily productivity was calculated as the difference between the AFDW

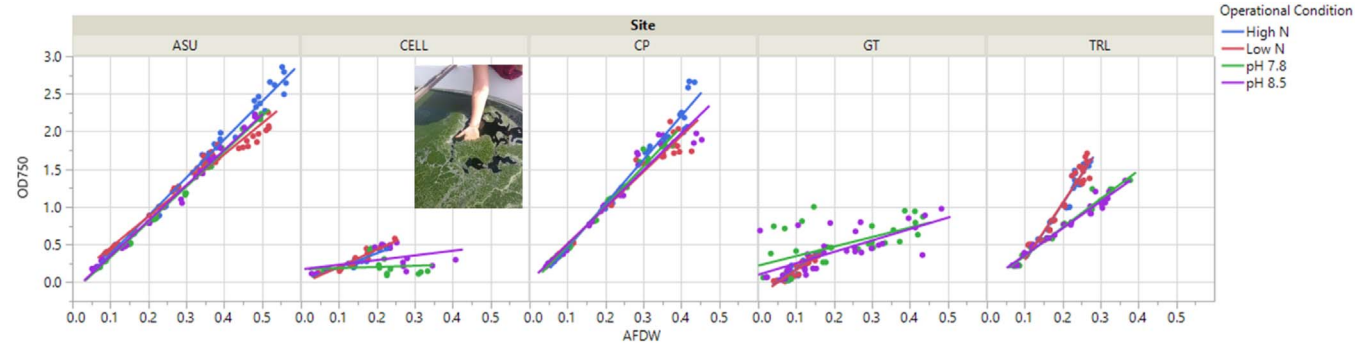

Fig. 6. Correlation between OD_{750} absorbance and measured AFDW (g L^{-1}) for each site. Inset for CELL shows a visual of the spontaneous flocculation event that took place, terminating the experiment.

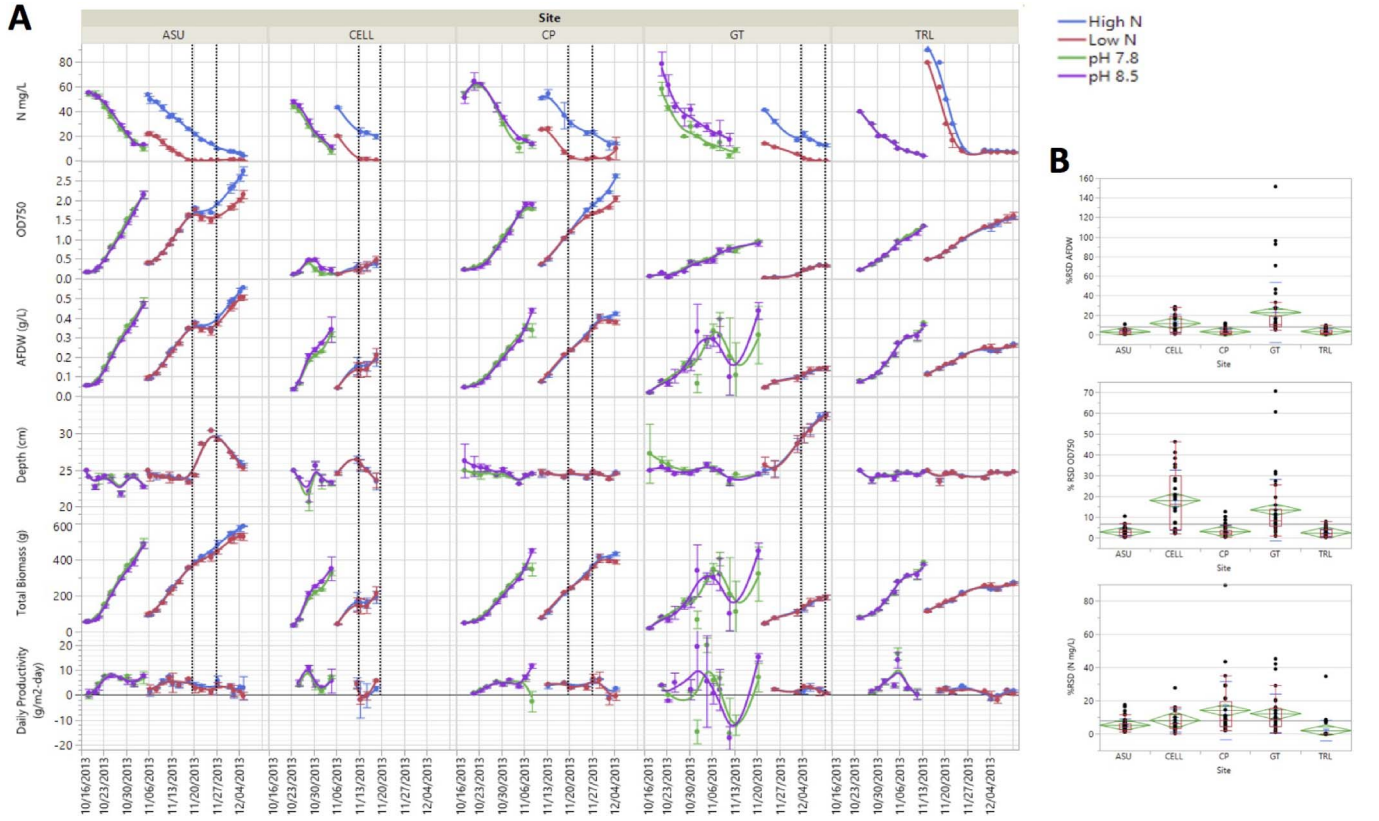

Fig. 7. Overview of the validation UFS data. (A) Data collected over the course of approximately 8 weeks (October 16–December 4, 2013) of cultivation of *N. oceanica* KA32 relative to two physiological study experiments (pH and initial nitrogen concentration as described in the text and reflected by colors). Parameters shown (top to bottom): nitrogen concentration (N, mg L^{-1}), OD_{750}, AFDW (g L^{-1}), pond depth (cm, reflecting rainfall), total biomass accumulated in the pond (g), and average daily productivity (g m^{-2} day^{-1}) calculated from the differential in AFDW each day. Dashed black vertical lines show a one-week window after nitrogen was fully depleted from the media. (B) Relative standard deviation of measurements of AFDW, OD_{750}, and nitrogen as box-and-whisker plots as described for Fig. 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(t2) and AFDW (t1) multiplied by the pond volume (L). This sum was divided by the 4.2 m^2 pond surface area divided by the duration between the two AFDW measurements (days) (Eq. (1)).

$$\text{Daily productivity} = \frac{(\text{AFDW}_{t2} - \text{AFDW}_{t1}) \times \text{Volume (L)}}{\text{Pond Area (m}^2) \times (\text{days}_{t1} - \text{days}_{t2})} \quad (1)$$

This metric represents the accumulation of algal biomass within the pond in terms of g m^{-2} day^{-1}. For the first experiment having different pH set points, the calculated productivity was similar between the ponds with differential treatment and fluctuated between 5 and 10 g m^{-2} day^{-1} for the last two weeks of the experiment. However, for CP, a similar trend was observed, though with a distinct separation between the pH treatments for the last three days of the experiment. It was not clear what caused this distinction, but we believe that this differential in daily productivity for the final time point was dominated by the decrease in AFDW of the final time point in the low pH ponds (pH 7.8) due to increased contamination pressure.

When the productivity data between the sites and experiments are combined, distinct trends in calculated areal productivity were found (Fig. 8A). Consistent with the data shown in Fig. 7, during the first pH experiment, two sites (ASU and CP) had higher productivities than the other three, averaging 5.5 to 6.5 g m^{-2} day^{-1}, with the lowest productivity observed for GT and TRL, on average 3 g m^{-2} day^{-1}. The second initial nitrate concentration experiment showed a significant drop in productivity, which was mainly explained by the late-November to December timeframe of the experiment, where the reduced overall light availability and ambient temperature also reduced productivity. Significant differences in productivity were observed between the high and low nitrate ponds with an approximately 10–15% decrease in the low nitrate ponds at ASU and CP. The other sites did not exhibit this distinction between the treatments, but also showed calculated areal productivities less than 2 g m^{-2} day^{-1}. We conclude that the rate of biomass accumulation at these sites was not high enough for the cells to experience true nitrogen limitation. Interestingly, during the initial nitrogen concentration experiment; even though the ponds at TRL were sheltered in a temperature controlled greenhouse and maintained a relatively stable temperature that was warmer than ponds at GT, near the maximum temperature at CP, and about the mid-point of the temperature for ASU; the data shows that those ponds were less productive. Looking at energy input (i.e. available PAR) during this time, it was also much lower than at other sites. Thus available solar energy appeared to be the primary driver for areal productivity as even stably maintained warm pond water temperatures could not match areal productivity with outdoor ponds having pond water temperatures that fluctuated by up to 10 °C daily yet had 1.3 to 2× greater solar input.

Biochemical composition data were collected for each of the two experiments (Fig. 8B). These data and the underlying standardization effort are discussed in depth in an accompanying manuscript due to the complexity of techniques and the distinct standardization [27]. In brief, the results of the biomass compositional analysis did not vary significantly during the first pH experiment, where on average, between 9 and 15% lipids as FAME were measured. Upon nitrogen depletion during the second experiment, a dramatic increase in FAME lipids were observed for ASU and CP, where for both sites, the average lipid content reached over 30% on an AFDW basis. The overall carbohydrate content at all sites ranged between 6 and 13% of the biomass for the duration of both experiments. This shift in FAME lipids content was most pronounced for ASU and CP where growth was the most stable and robust for the duration of the experiment. Protein content typically

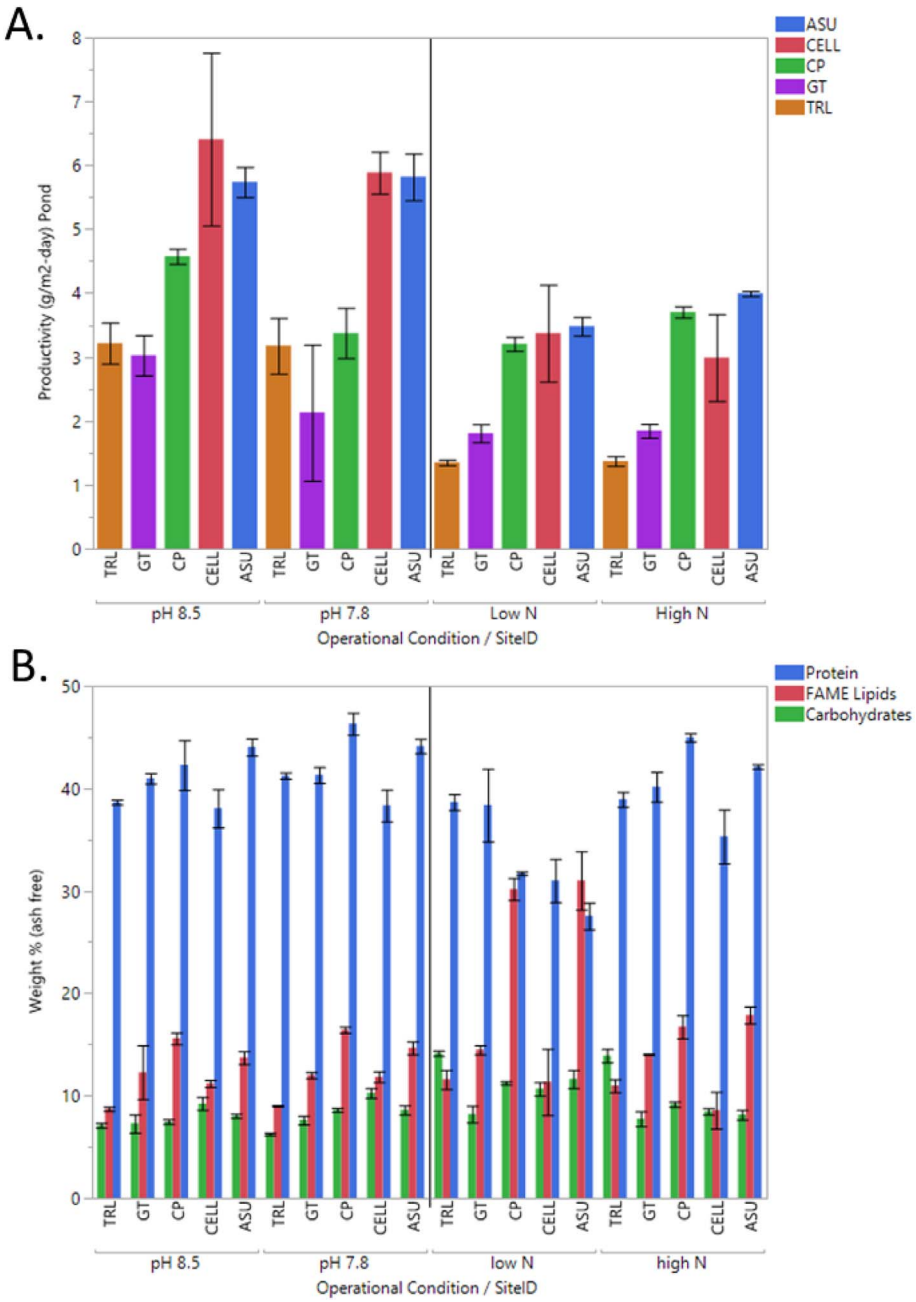

Fig. 8. Calculated average areal productivity and biomass composition for the four experimental variables tested in open ponds. (A) Calculated average areal productivity (g m^{-2} day^{-1}) derived from daily AFDW measurements. The data shown are the mean ± standard deviation of three replicate ponds for each experimental condition. (B) Protein, FAME lipids, and carbohydrate composition of the harvested algal biomass at the conclusion of each experiment. The data shown are the mean ± standard deviation of three replicate ponds for each experimental condition.

ranged from 45% to approximately 25% at the later time points during nitrogen stress showing the typical inversely correlated shift in lipid content, which is in agreement with literature reports on carbon and nitrogen metabolism in algae [25,31–35]. We noticed during the compositional data analysis for TRL that the alga used for inoculation was not *N. oceanica* KA32, but rather a *Nannochloris* species. The *N. oceanica* KA32 species that we were working with has a distinct fatty acid profile as detailed in our accompanying manuscript [27] always contains C20:5 (eicosapentaenoic acid) [26]. Thus, we were able to confirm that the alga present in the ponds was not *N. oceanica* KA32 by virtue of its fatty acid profile. Furthermore, the alga was confirmed to be *Nannochloris* sp. by 18S sequencing (H. Gerken, ASU, personal communication). Because both ponds for the N experiment were inoculated with the same high (100 ppm) concentration of nitrate, the increase in carbohydrates observed is not as high as could have been observed if there was an actual nutrient depletion event ongoing. *Nannochloris* has previously been demonstrated to favor the accumulation of carbohydrates upon nutrient depletion which is distinct from *Nannochloropsis* [36].

4. Conclusions

This ATP^3 consortium was set up with the goal of integrating algal cultivation systems with feedstock characteristics and ultimately relating this to technical targets for biofuels and bioproducts. Within the successful establishment of this testbed framework, the validation UFS was carried out as an operational baseline to understand the variability and challenges of the coordinated operation of a distributed testbed

network. The data presented here summarize the process that the ATP^3 consortium implemented to align very different geographical sites with the same cultivation experimental set up. We demonstrated that by using rigorously developed experimental and data quality review protocols across a standardized framework of algal cultivation sites, productivity data was highly reproducible within a testbed site and between replicate ponds and ultimately can be meaningfully compared both within and across testbed sites. Based on this validation UFS experiment, we found distinct characteristics across the sites, with ASU and CELL exhibiting the highest calculated areal productivity, and the lowest across all experiments at GT, with minor differences observed between the different experimental treatments. It was clear after this validation UFS experiment that the operational, data collection, and quality review procedures underpinning these experiments was labor-intensive. In particular, much discipline was necessary to keep up with the demands of maintaining probes for the automated sensors and carrying out manual sampling, data collection, and sample processing protocols. The knowledge gathered in the successful operation of the testbed network during this validation UFS was carried forward to a multi-year UFS study designed to look at the seasonal, geographical, and climatic differences in relation to algal biomass production and harvest yields under different operational strategies using the same algal species across the testbed network. One ultimate goal for the ATP^3 network is to integrate the meteorological parameters with areal productivity and composition of the biomass in a manner that allowed for predictive modeling and such an integration will be reported in a future manuscript on the long-term implementation of the UFS experimental design.

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