Using algal microcosms in introductory biology lab. II: The influence of biodiversity on ecosystem productivity

Michael J. Wise[1,2*] and Rachel Collins[2]

[1]Environmental Studies Program, Roanoke College, Salem, VA 24153

[2]Department of Biology, Roanoke College, Salem, VA 24153

*Corresponding author: wise@roanoke.edu

Abstract

Because the escalating loss of species is one of the most serious environmental crises we face, it is vital that students of biology comprehend and can communicate the roles that biodiversity plays in the functioning of ecosystems. Here, we report on a laboratory experiment that has been highly successful in our introductory biology courses at demonstrating the role of biodiversity in the productivity of ecosystems. Students created microcosms consisting of six species of phytoplankton grown separately (monocultures) and in mixtures (polycultures) in 50-mL beakers. On average, monocultures were less productive than three-species polycultures, which in turn were less productive than six-species polycultures. However, the productivity of the polycultures was generally no greater than the productivity of the most productive monocultures—a result that suggests a lack of positive ecological interactions (e.g., facilitation or niche complementarity). In contrast, there was some evidence of negative interactions among species (e.g., predation and allelopathy) that caused some polycultures to be less productive than monocultures of their constituent species. Performing this structured-inquiry experiment provided students with an opportunity to apply aspects of the scientific method in a non-trivial manner to address issues of relevance to both basic ecology principles and applied disciplines with economic significance.

Keywords: biodiversity, microcosms, niche complementarity, phytoplankton, productivity, species richness, structured inquiry, transgressive overyielding.

Introduction

The number of species present in an ecosystem (i.e., its species "richness") is important from both a basic science and an applied perspective. Specifically, species richness can influence the stability and productivity of an ecosystem, which in turn affect the goods and services that ecosystems provide to benefit humans (Balvanera et al., 2006; Cardinale, 2011; Cardinale et al., 2011; Hooper et al., 2012; Gross et al., 2014; Liu, 2016). A basic question is whether ecosystems composed of many species tend to be more productive than ecosystems composed of fewer species (Duffy, 2009; Willig, 2011; Stockenreiter et al., 2012; Yuan et al., 2015). For managed ecosystems, a common question is which combinations of species should be propagated in order to maximize biomass production for food or fuel.

The alteration of species richness can have a range of effects on the productivity of an ecosystem, depending on the niche requirements of the species and how the species interact. If two species in an environment use the same resources in the same manner, then their combined productivity when grown together (in a polyculture) is likely to be the average of their productivities when grown separately (in monocultures). However, biological interactions may lead to a phenomenon called "complementarity," such that the total productivity in a polyculture is greater than the productivities of any of the component species in monoculture (Cardinale et al., 2007; Nalley et al., 2014; Brooker et al., 2015; Wendling et al., 2017). For instance, if the two species do not use the same resources in the same manner, then the two species in a polyculture may use of the resources in an ecosystem more completely—a phenomenon known as "niche complementarity." In addition, if one species is able to acquire a resource in abundance that the other species cannot (e.g., one species can fix nitrogen) or otherwise improves the habitat for another species, then the productivity of the polyculture is also likely greater than either monoculture—a phenomenon called "facilitation" (Brooker et al., 2015).

Other interspecific interactions can lead a polyculture to be less productive than the component monocultures. For instance, species may interfere

with each other's ability to obtain resources—either physically or through the release of toxic or inhibitory chemicals (i.e., allelopathy)—more strongly than they compete with individuals of their own species. Thus, the productivity of a polyculture can be lower than the mean productivity of the component species in monocultures (Nalley et al., 2014; Newby et al., 2016).

In addition to biological interactions, stochasticity involved in "choosing" which species will make up a community in an ecosystem can affect how richness relates to productivity. Specifically, a polyculture that happens to include a particularly productive species is likely to be more productive than the average monoculture. In contrast, a polyculture that is dominated by species of low productivity will tend to be less productive than the average monoculture. These phenomena are often called "sampling" (or "selection") effects to emphasize the fact that they result from the vagaries of community assembly in nature or in the design of an experiment (Tilman et al., 2001; Fridley, 2002; Fox, 2005; Stockenreiter et al., 2012; Nalley et al., 2014; Weis, 2016).

One can gain insight as to whether a relationship between species richness and productivity is driven by complementarity or by sampling effects by inspecting the productivity patterns in monocultures and polycultures (Schmid et al., 2008; Wang et al., 2013). In particular, if the productivity of a polyculture is greater than the productivity of every monoculture (a pattern called "transgressive overyielding"), then complementarity is most likely involved (Hector et al., 1999; Shurin et al., 2013). In contrast, if the productivity of a polyculture is lower than every monoculture (i.e., "transgressive underyielding"), then substantial inhibition or interference among the species is likely to be the cause. Sampling effects can lead to overyielding or underyielding relative to the mean productivity of the monocultures, but sampling effects alone would not cause the pattern to be transgressive. Sophisticated experimental designs and statistical analyses can be used partition the influence of sampling and complementarity effects (Loreau & Hector, 2001; Fox, 2005; Yuan et al., 2015). However, a basic appreciation of the biological interactions at play can be obtained by a simple assessment of whether patterns of overyielding or underyielding are transgressive (Hector et al., 1999; Tilman et al., 2001; Shurin et al., 2013).

Some of the most comprehensive work on the diversity-productivity relationship has been performed in grassland ecosystems (Tilman et al., 1997; Hector et al., 1999; Dukes, 2001; Tilman et al., 2001; Balvanera et al., 2006; Grace et al., 2007). For example, in a seminal paper summarizing the results of many years of study, Tilman et al. (2012) reported that the positive effect of species richness on the productivity of grassland ecosystems was greater in magnitude than the effects of nitrogen levels, drought, carbon dioxide levels, herbivores, and fire. In recent years, more attention has been focused on investigating the effect of species richness on productivity of phytoplankton communities (i.e., floating aquatic, photosynthetic micro-organisms, or "algae") (Weis et al., 2008; Striebel et al., 2009; Weis, 2016). Phytoplankton are appealing to researchers because their communities can be experimentally manipulated in laboratory microcosms that allow a large degree of replication. In addition, the potential for phytoplankton to serve as a source of biofuels is making such studies more valuable from an applied perspective (Smith et al., 2009; Stockenreiter et al., 2012; Shurin et al., 2013; Shurin et al., 2014; Liu, 2016; Newby et al., 2016; Jackrel et al., 2018).

This paper reports on a laboratory experiment conducted by an introductory majors' biology course in which students created microcosms of phytoplankton to investigate the effect of species richness on ecosystem productivity. Prior to the experiment, students read and discussed two scientific journal articles related to biodiversity and productivity—one on grassland ecosystems (Tilman et al., 2012) and the other on phytoplankton (Weis, 2016). After a lecture on productivity and a brief overview of the use of algal microcosms, students met with their lab groups (3-4 students each) to formulate research questions, generate hypotheses, and brainstorm possible experimental designs. Then through a whole-class discussion, the instructors guided students to an agreed-upon set of questions and experimental protocols to address the questions. Specifically, students constructed glass-beaker microcosms in which phytoplankton were grown as monocultures, three-species polycultures (3-SP's), and six-species polycultures (6-SP's), and the productivity of the microcosms was measured after two weeks of growth. Students addressed three main experimental questions: 1) Did the species differ from each other in productivity when grown in monocultures? 2) Did the 3-SP's or 6-SP's differ in productivity from each other or from the monocultures of the component species? and 3) Was

there any evidence of transgressive overyielding or underyielding, suggesting the agency of complementarity or inhibition, respectively?

The intended learning outcomes for this project were that students should be able to do the following: 1) communicate the importance of species richness to the productivity of an ecosystem; 2) employ aspects of the scientific method in a structured-inquiry experiment to address an important ecological question; 3) use Excel to perform statistical analyses and construct professional-quality graphs; and 4) interpret and communicate the results and their broader implications. Their achievement of these learning outcomes was assessed through a formal lab report, written in the format of an article for an ecological journal.

Materials and Methods

Course overview

The microcosm experiments described here (and in a companion paper: Wise & Collins, this issue) are the principal laboratory activities of the introductory biology course BIOL 180 (Exploring Biological Diversity) at Roanoke College, a selective liberal arts institution of ~2,000 students in Salem, VA, USA. BIOL 180 is one of a sequence of three introductory courses for Biology majors, but it is also taken by some non-majors for whom this is their only biology course. Versions of these microcosm experiments have been used in 16 sections of BIOL 180 since 2015. This course meets for three two-hour periods per week, and the class is capped at 24 students. The design and data reported in this paper are from a version of the experiment used in the fall semester of 2018 in a section with 14 students.

Phytoplankton Species

Six freshwater phytoplankton species across six different genera were chosen for inclusion in the microcosm experiment (Table 1). All of these species grow well in laboratory conditions and are commercially available (Carolina Biological Supply Company, Burlington, NC, USA). This set of species included four green algae, two of which are charophytes of the family Desmidaceae, and two of which are chlorophytes of two different families. The set also included one diatom and one euglenozoan. Each species was maintained in a separate 250-mL beaker containing 200 mL of growth medium, which consisted of a 1:1 ratio of autoclaved tap water to deionized water with one 20-mL tube of AlgaGro® Concentrated Medium (Carolina Biological Supply Company) per liter of water. These stock-culture beakers were covered with cellophane wrap with five small ventilation holes (made with dissecting needles) and were maintained on a light rack of constant fluorescent light for one week prior to the initiation of the microcosm experiment. The light was provided by four wide-spectrum tubes (F40 PL/AQ-ECO bulbs, General Electric) mounted ~40 cm above the shelf.

Microcosms

The class was split into six groups of students, and each group was assigned to prepare a set of 10-11 microcosms in 50-mL glass beakers with 30 mL of growth medium (for a class-wide total of 62 microcosms). Each set comprised six monocultures (one for each species), one polyculture of all six species (6-SP), and three or four polycultures of three species (3-SP's). In total, the experiment consisted of 36 monocultures, six 6-SP's, and 20 3-SP's (one for each of the 20 different three-species combinations). Using graduated cylinders, students added 30 mL of growth medium to each beaker. Then using 10-mL graduated pipettes, they added 6 mL of culture from a single species into each monoculture beaker, 1 mL of culture from each of the six species into their 6-SP beaker, and 2 mL of cultures from each of three designated species into their 3-SP beakers (Appendix 1.) Each stock solution was designated a single pipette, conspicuously labeled with the genus name. To prevent cross-contamination, students were instructed to double-check to make sure they only used the pipette whose label matched the label on

Table 1.

Taxonomic information for the six phytoplankton species included in this study

Genus	Superkindom[1]	Phylum/Division	Family
Ankistrodesmus	Archaeplastida	Chlorophyta	Selenastraceae
Cosmarium	Archaeplastida	Charophyta	Desmidaceae
Euglena	Excavata	Euglenozoa	Euglenaceae
Navicula	Stramenopila	Ochrophyta	Naviculaceae
Scenedesmus	Archaeplastida	Chlorophyta	Scenedesmaceae
Staurastrum	Archaeplastida	Charophyta	Desmidaceae

[1] Eukaryotic superkingdoms are as designated in Morris et al. (2016).

the stock culture before they drew samples.

Students covered their microcosms with cellophane wrap to prevent evaporation, secured the wrap with a rubber band, and punched three small ventilation holes in the wrap using dissecting needles. The microcosm beakers were placed on a tray on the light rack and housed in the same conditions as the stock cultures were for 14 days. The beakers were gently shaken daily to prevent permanent settling of cells on the bottom.

Productivity Measurements

The (net) productivity of a trophic level can be defined as the amount of new organic material produced for all the organisms of that trophic level per unit area (or volume), per unit time. In this experiment, all microcosms were the same total volume, and all had been growing for the same duration. Therefore, to compare the relative productivity of our microcosms, we can factor out volume and time and simply compare the amounts of organic material produced. We used spectrophotometry as a quick and precise method to estimate relative productivity of our microcosms because the denser the cells are in a microcosm (due to greater numbers or sizes of cells), the more light will be absorbed by a sample from the microcosm.

Prior to measuring absorbance for a microcosm, students used a plastic dropper to mix and evenly suspend the phytoplankton cells in the beaker. They then quickly filled the sample tube and put it into their spectrophotometer to take a measurement before the cells settled. The spectrophotometers (Genesys 20; ThermoFisher Scientific, Waltham, MA, USA) were set to a wavelength of 750 nm (Rodrigues et al., 2011; Shurin et al., 2014). The spectrophotometers were blanked prior to each absorbance measurement using a tube with growth medium (no phytoplankton). (Absorbance data for the 62 samples are in the Appendix.)

Data Analysis

We addressed the question of whether productivity differed among the six species in two steps. We first performed a two-way analysis of variance (ANOVA) with absorbance of the monocultures as the response variable and species as one predictor variable. The second predictor was a block (i.e., student group), which accounted for potential differences in absorbance due to such elements as procedural inconsistencies among student groups and differences in calibration among the spectrophotometers. Block was considered a random-effects factor, and the ANOVA was performed using Minitab 14 (Minitab, LLC, Statesville, PA, USA). We then performed pairwise comparisons between the mean productivities of the monocultures for each species—as well as the means of the 3-SP's and 6-SP's—using Tukey tests with an experiment-wise alpha of 0.05.

In addition to the Tukey tests described above, we asked whether polycultures differed in productivity from monocultures using paired t-tests of absorbance values. Paired t-tests were more appropriate than standard two-sample t-tests because pairing allowed us to take into account potential block differences in absorbance values. Moreover, pairing allowed us to compare the absorbances of each polyculture with only the monocultures of species that constituted that polyculture. For instance, the absorbance of a polyculture that contained *Ankistrodesmus*, *Cosmina*, and *Euglena* would be compared with the mean absorbances of the monocultures of these same three species (in the same block). After calculating the 20 mean-absorbance values to pair with the 20 3-SP values, students performed a paired t-test using Excel. They also performed a second paired t-test to compare the productivity of the six 6-SP's to the monocultures, pairing each 6-SP value with the mean of the monocultures from the same block.

Students were given guidance to examine the patterns of overyielding and underyielding more closely using graphical and mathematical methods (e.g., Cardinale et al., 2007; Weis et al., 2008; Gamfeldt et al., 2014; Shurin et al., 2014; Weis, 2016). Here, we present log-response ratios, modelled after Gamfeldt et al. (2014) and Shurin et al. (2014), that allow discrimination between simple and transgressive overyielding. For each of the 26 polycultures, we calculated both a net biodiversity effect (NBE) and an overyielding (OY) metric, which respectively were $\log_{10}$ ratios of polyculture absorbance (P) to the average monoculture absorbance (M_{ave}) or the maximum monoculture absorbance (M_{max}) for the species constituting the polyculture:

$$NBE = log\left(\frac{P}{M_{ave}}\right) \qquad OY = log\left(\frac{P}{M_{max}}\right)$$

A value of NBE > 0 would indicate that the polyculture was more productive than the average of the monocultures of the species that constituted the polyculture, and if OY > 0, then the overyielding was transgressive.

Results

The productivities of the six phytoplankton species differed significantly, as determined by the ANOVA of absorbance values (Table 2) and the Tukey tests (Fig. 1). Student group (i.e., block) also had a significant influence on the absorbance measurements. However, the amount of variation explained by student-group differences was small (< 4% as much as that explained by the identity of the phytoplankton species).

The productivities of the polycultures tended to be greater than the average productivities of the monocultures. Specifically, the mean absorbance of the 6-SP's was roughly twice as great as the mean for monocultures, and the mean absorbance for the 3-SP's was 1.4 times as great as the monoculture mean (Fig. 1). In addition, the paired t-tests indicated that the productivities of both the 6-SP's (t_5 = 9.59, P = 0.0002) and the 3-SP's (t_{19} = 3.49, P = 0.002) were significantly greater than the mean productivities of the monocultures.

The net biodiversity effects tell a similar story regarding the relative productivities of polycultures and monocultures (Fig. 2A). Specifically, all of the 6-SP's and 70% of the 3-SP's had a positive NBE value. Notably, six of the 3-SP's had a negative NBE, with two of these polycultures showing substantially lower productivity than the means of the monocultures of the same species.

Table 2.

Summary of ANOVA results for differences in light absorbance (productivity) among genera of phytoplankton. Student group was considered a random-effects factor.

Source of variation	df	Mean square	F-ratio	P-value
Student group	5	0.002617	5.74	0.001
Genus	5	0.073053	160.16	<0.001
Error	25	0.000456		

There was very little indication of transgressive overyielding by polycultures in this experiment. Note that monocultures of *Ankistrodesmus* were just as productive as the average 6-SP, and were significantly more productive than the average 3-SP (Fig. 1). On a finer level, only four of the 3-SP's and one of the 6-SP's had a positive OY value, and these magnitudes were quite close to zero (Fig. 2B). One microcosm showed transgressive underyielding—the 3-SP containing *Euglena*, *Navicula*, and *Staurastrum*.

Discussion

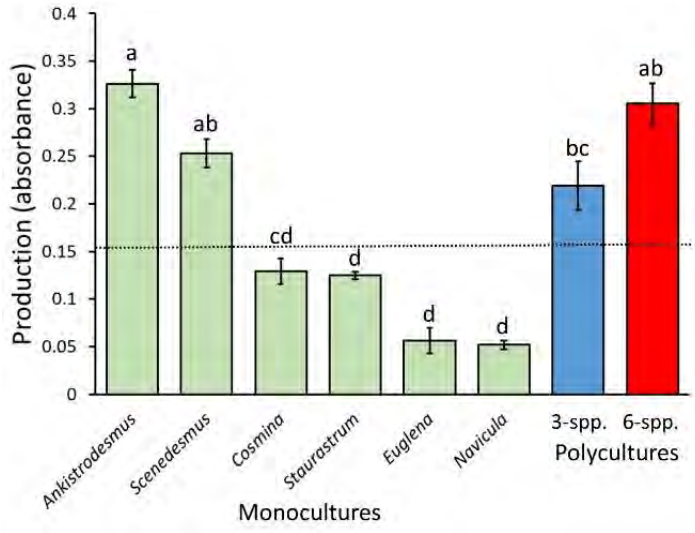
Interpretation of Experimental Results

There was a substantial range of variation in productivities of the phytoplankton species in monocultures, and taxonomic affinity seems to explain some of this variation. In particular, the two chlorophytes had the greatest productivity, with *Ankistrodesmus* being significantly more productive than all species besides *Scenedesmus*. The two charophytes (*Cosmina* and *Staurastrum*) were significantly less productive than the chlorophytes. The two least-productive species were the diatom (*Navicula*) and *Euglena*. If one were interested in cultivating one of these species for biofuel production, these results suggest that *Ankistrodesmus* holds the most promise strictly from the standpoint of productivity. The more interesting question addressed by this study is whether the productivity of the system can be increased by using different combinations of species, rather than just a monoculture.

This study produced solid evidence that polycultures of phytoplankton are more productive, on average, than monocultures. Moreover, the results suggest that the greater the species richness, the greater the average productivity is likely to be. This laboratory result is likely to hold in natural ecosystems as well, if only for the fact that the more species are present in a community, the more likely it is that the community will contain one or more highly productive species. This is the essence of a positive sampling (i.e., selection) effect. The next question is whether the positive richness-productivity relationship observed in this study is due only to a sampling effect, or whether there is evidence of complementarity, which would suggest the action of more interesting biological phenomena.

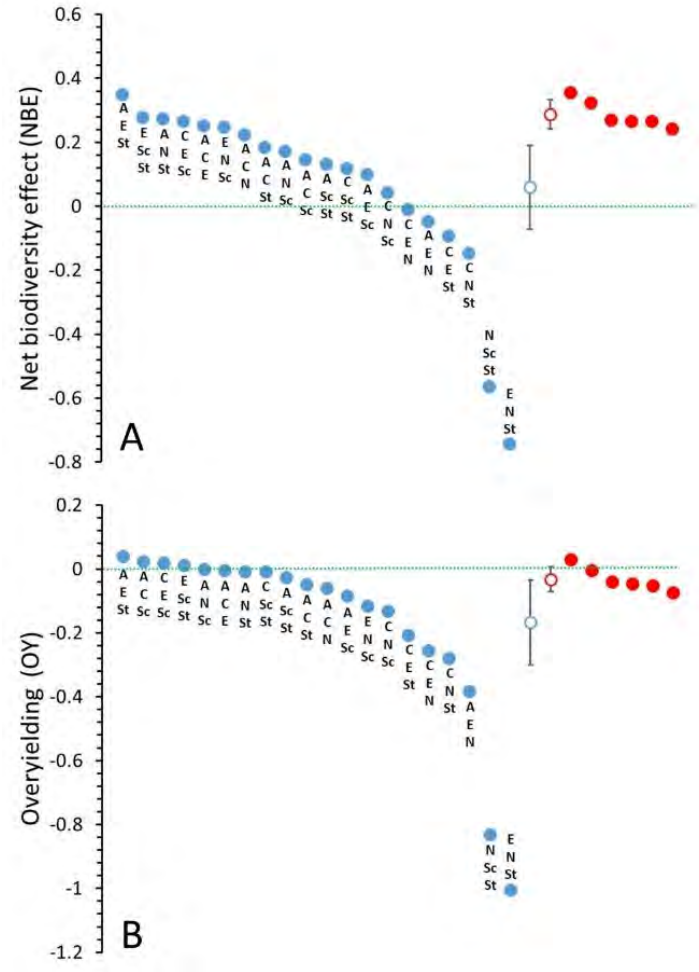
Although there was good evidence of polycultures being more productive than the means of the monocultures, the productivity of the polycultures did not exceed the productivity of every one of the species in monoculture. In other words, there was evidence of overyielding in the polycultures, but this overyielding was not of the transgressive variety. Therefore, it would appear that sampling effects alone can explain why the polycultures were more productive than the average monocultures.

Figure 1.



Relative productivity of six genera of phytoplankton, measured by light absorbance at 750 nm. Columns and bars represent means +/- one standard error. The horizontal dashed line indicates the overall mean absorbance for the monocultures. There were six replicates per monoculture (green bars), 20 replicates for all possible combinations of 3-species polycultures (blue bar) and six replicates of 6-species polycultures (red bar). Genera that share lower case leters above the bars did not differ significantly from each other in relative productivity, based on Tukey tests with experiment-wise rate of 0.05.

Figure 2.



Comparisons of productivities of polycultures and monocultures. A. A positive NBE value indicates that the productivity of the polyculture was greater than the mean of the productivities of the monocultures of the genera that constituted the polyculture. B. A positive OY value indicates that the productivity of the poly culture was greater than the productivities of each of the monocultures of the genera that constituted the polyculture (i.e., the overyielding was transgressive). Filled blue and red circles represent values for 3-species and 6-species polycultures, respectively. Letters above and below the blue circles are abbreviations for the genera that composed the polycultures. The blue and red open circles represent the mean values for the 20 3-species polycultures and the six 6-species polycultures, respectively, and the bars indicate the 95% confidence intervals for the means.

The overall picture that emerged was that there was little compelling evidence of niche complementarity or evidence that any of the species improved the production of any of the other species. However, there was evidence that the productivities of some species were actually reduced by the presence of other species. Specifically, six of the 3-SP's had negative NBE values, and two had highly negative OY values. These patterns suggest antagonistic interactions beyond simple resource competition.

In our microcosms, the 3-SP's containing *Euglena* tended to have especially low NBE and OY values—unless either *Ankistrodesmus* or *Scenedesmus* was also present. The most likely explanation for these low values is the fact that *Euglena* is not strictly autotrophic. In particular, *Euglena* is known to consume individuals of some species of diatoms and green algae. Thus, the 3-SP's that contained *Euglena* and some combination of the diatom *Navicula* and the two charophyte algae (*Cosmina* and *Staurastrum*) could have been less productive than the mean monocultures simply because *Euglena* was consuming some of the biomass produced by these species. It would seem that the chlorophyte algae were less suitable as food items for *Euglena*—perhaps simply due to their spiny bodies.

The 3-SP's that contained *Navicula* were even less likely to display a positive NBE or OY value than were the 3-SP's containing *Euglena*. While diatoms like *Navicula* are not heterotrophic, they are known to produce allelopathic chemicals that poison not only herbivores, but other phytoplankton sharing an environment with the diatoms (Ianora & Miralto, 2010; Pichierri et al., 2017). In our experiment, polycultures with *Navicula* may have had lower productivity than expected because *Navicula* produced toxins that poisoned individuals of the other species in polyculture. While these interpretations of the results involving *Navicula* and *Euglena* are speculative, potential heterotrophy and allelopathy, combined with their low productivity in monocultures, would seem to eliminate these species as candidates for biofuel production.

Pedagogical Considerations

The main caveat regarding the experimental questions is that they may have seemed a bit too esoteric for students in an introductory biology course. In particular, the distinction between sampling effects and complementarity, or the significance of the difference between simple overyielding and transgressive overyielding were a bit nuanced for some students to appreciate. To prepare students for the more esoteric concepts, we spent the greater part of two class meetings discussing these concepts from required readings prior to the experiment (Tilman et al., 2012; Weis, 2016). We spent another class period discussing the students' results in terms of these concepts. In past iterations of this experiment, we have left out these more advanced concepts and simply examined overall relative productivity differences between monocultures and polycultures. Both strategies have their advantages.

The flexibility in terms of the target level of sophistication is one of the valuable features of this experiment. For instance, instructors can have just as successful experiences with this experiment if they choose to leave out the statistical tests and focus on qualitative differences in productivity if that strategy better matches the experience level of their students.

Conclusion

The experiment provided a hands-on opportunity for introductory biology students to investigate a set of questions that are significant from both a basic ecology and applied science perspective. The broad interest in issues of biodiversity and productivity provided ample opportunities for students to engage with relevant scientific literature. Importantly, the study was hypothesis driven, and because the results were not obvious to the students prior to the experiment, the study provided an authentic application of several steps of the scientific method. The data generated in the experiment allowed opportunities for graphing and statistical analyses of a range of sophistication. Finally, we have found the results to be highly consistent and repeatable across years and sections of the course.

Acknowledgments

We thank the students of Roanoke College who participated in collecting data, the RC Biology Department for logistical support, an anonymous reviewer for helpful comments on the manuscript, and S.E. Wise for carefully editing the manuscript.

Appendix 1.

Contents of microcosms and absorbance data after two weeks of growth.
The Blocks represent student groups. Each group was responsible for six monocultures, one 6-species polyculture, and three or four 3-species polycultures.

Beaker No.	Block	Genera per Microcosm	Volumes of Stock Solutions per Genus	Abs.
1	A	one	6 mL *Ank.*	0.366
2	A	one	6 mL *Cos.*	0.154
3	A	one	6 mL *Eug.*	0.088
4	A	one	6 mL *Nav.*	0.052
5	A	one	6 mL *Sce.*	0.310
6	A	one	6 mL *Sta.*	0.143
7	A	six	1 mL each genus	0.391
8	A	three	2 mL *Ank., Cos.,* & *Eug.*	0.362
9	A	three	2 mL *Ank., Cos.,* & *Nav.*	0.319
10	A	three	2 mL *Ank., Cos.,* & *Sce.*	0.387
11	B	one	6 mL *Ank.*	0.370
12	B	one	6 mL *Cos.*	0.154
13	B	one	6 mL *Eug.*	0.084
14	B	one	6 mL *Nav.*	0.058
15	B	one	6 mL *Sce.*	0.273
16	B	one	6 mL *Sta.*	0.127
17	B	six	1 mL each genus	0.327
18	B	three	2 mL *Ank., Cos.,* & *Sta.*	0.332
19	B	three	2 mL *Ank., Eug.,* & *Nav.*	0.153
20	B	three	2 mL *Ank., Eug.,* & *Sce.*	0.305
21	C	one	6 mL *Ank.*	0.286
22	C	one	6 mL *Cos.*	0.120
23	C	one	6 mL *Eug.*	0.013
24	C	one	6 mL *Nav.*	0.042
25	C	one	6 mL *Sce.*	0.248
26	C	one	6 mL *Sta.*	0.121
27	C	six	1 mL each genus	0.257
28	C	three	2 mL *Ank., Eug.,* & *Sta.*	0.314
29	C	three	2 mL *Ank., Nav.,* & *Sce.*	0.286
30	C	three	2 mL *Ank., Nav.,* & *Sta.*	0.281

Beaker No.	Block	Genera per Microcosm	Volumes of Stock Solutions per Genus	Abs.
31	D	one	6 mL *Ank.*	0.300
32	D	one	6 mL *Cos.*	0.117
33	D	one	6 mL *Eug.*	0.030
34	D	one	6 mL *Nav.*	0.052
35	D	one	6 mL *Sce.*	0.208
36	D	one	6 mL *Sta.*	0.116
37	D	six	1 mL each genus	0.253
38	D	three	2 mL *Ank., Sce.,* & *Sta.*	0.283
39	D	three	2 mL *Cos., Eug.* & *Nav.*	0.065
40	D	three	2 mL *Cos., Eug.* & *Sce.*	0.218
41	E	one	6 mL *Ank.*	0.307
42	E	one	6 mL *Cos.*	0.156
43	E	one	6 mL *Eug.*	0.084
44	E	one	6 mL *Nav.*	0.069
45	E	one	6 mL *Sce.*	0.223
46	E	one	6 mL *Sta.*	0.120
47	E	six	1 mL each genus	0.279
48	E	three	2 mL *Cos., Eug.* & *Sta.*	0.097
49	E	three	2 mL *Cos., Nav.* & *Sce.*	0.165
50	E	three	2 mL *Cos., Nav.* & *Sta.*	0.082
51	E	three	2 mL *Cos., Sce.* & *Sta.*	0.219
52	F	one	6 mL *Ank.*	0.328
53	F	one	6 mL *Cos.*	0.074
54	F	one	6 mL *Eug.*	0.004
55	F	one	6 mL *Nav.*	0.038
56	F	one	6 mL *Sce.*	0.258
57	F	one	6 mL *Sta.*	0.121
58	F	six	1 mL each genus	0.325
59	F	three	2 mL *Eug., Nav.* & *Sce.*	0.198
60	F	three	2 mL *Eug., Nav.* & *Sta.*	0.012
61	F	three	2 mL *Eug., Sce.* & *Sta.*	0.265
62	F	three	2 mL *Nav., Sce.* & *Sta.*	0.038

.

References

Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.S., Nakashizuka, T. et al. (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters, 9, 1146-1156.

Brooker, R.W., Bennett, A.E., Cong, W.F., Daniell, T.J., George, T.S. et al. 2015. Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. New Phytologist, (206), 107-117.

Cardinale, B.J. (2011). Biodiversity improves water quality through niche partitioning. Nature, 472, 86-91.

Cardinale, B.J., Matulich, K.L., Hooper, D.U., Byrnes, J.E., Duffy, E., et al. (2011). The functional role of producer diversity in ecosystems. American Journal of Botany, 98(3), 572-598.

Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., et al. (2007). Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Sciences, USA, 104(46), 18123-18128.

Duffy, J.E. (2009). Why biodiversity is important to the functioning of real-world ecosystems. Frontiers in Ecology and the Environment, 7(8), 437-444.

Dukes, J.S. (2001). Productivity and complementarity in grassland microcosms of varying diversity. Oikos, 94, 468-480.

Fox, J.W. (2005). Interpreting the 'selection effect' of biodiversity on ecosystem function. Ecology Letters, 8, 846-856.

Fridley, J.D. (2002). Resource availability dominates and alters the relationship between species diversity and ecosystem productivity in experimental plant communities. Oecologia, 132, 271-277.

Gamfeldt, L., Lefcheck, J.S., Byrnes, J.E.K., Cardinale, B.J., Duffy, J.E., et al. (2014). Marine biodiversity and ecosystem functioning: what's known and what's next? Oikos 124: 252-265.

Grace, J.B., Anderson, T. M., Smith, M.D., Seabloom, E., Andelman, S.J., et al. (2007). Does species diversity limit productivity in natural grassland communities? Ecology Letters, 10, 680-689.

Gross, K., Cardinale, B.J., Fox, J.W., Gonzalez, A., Loreau, M., et al. (2014). Species richness and the temporal stability of biomass production: a new analysis of recent biodiversity experiments. American Naturalist, 183(1), 1-12.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., et al. (1999). Plant diversity and productivity experiments in European grasslands. Science, 286, 1123-1127.

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., et al. (2012). A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature, 486, 105-109.

Ianora, A., & MIRALTO, A. (2010). Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: a review. Ecotoxicology, 19(3), 493-511.

Jackrel, S.L., Narwani, A.,Bentlage, B., Levine, R.B., Hietala, D.C., et al. (2018). Ecological engineering helps maximize function in algal oil production. Applied and Environmental Microbiology, 84(15), 1-16.

Liu, J. (2016). Interspecific biodiversity enhances biomass and lipid productivity of microalgae as biofuel feedstock. Journal of Applied Phycology, 28, 25-33.

Loreau, M., & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. Nature, 412, 72-76.

Nalley, J.O., Stockenreiter, M., & Litchman,E. (2014). Community ecology of algal biofuels: complementarity and trait-based approaches. Industrial Biotechnology, 10(3), 191-201.

Newby, D.T., Mathews, T.J., Pate, R.C., Huesemann, M.H., Lane, T.W., et al. (2016). Assessing the potential of polyculture to accelerate algal biofuel production. Algal Research 19: 264-277.

Pichierri, S., Accoroni, S., Pezzolesi, L., Guerrini, F., Romagnoli, T., et al. (2017). Allelopathic effects of diatom filtrates on the toxic benthic dinoflagellate Ostreopsis cf. ovata. Marine Environmental Research, 131, 116-122.

Rodrigues, L.H.R., Arenzon, A., Raya-rodriguez, M.T., & Fontoura, N.F. (2011). Algal density assessed by spectrophotometry: A calibration curve for the unicelluar algae Pseudokirchneriella subcapitata. Journal of Environmental Chemistry and Ecotoxicology, 3(8), 225-228.

Schmid, B., Hector, A., Saha, P., & Loreau, M. (2008). Biodiversity effects and transgressive overyielding. Journal of Plant Ecology, 1(2), 95-102.Shurin, J., Mandal, S., & Abbott, R. L. (2014.) Trait diversity enhances yield in algal biofuel. Journal of Applied Ecology, 51, 603-611.

Shurin, J.B., Abbott, R.L., DeaL, M.S., Kwan, G.T., Litchman, E., et al. 2013. Industrial-strength ecology: trade-offs and opportunities in algal biofuel production. Ecology Letters,16(11), 1393-1404.

Smith, V.H., Sturm, B. S. M., Denoyelles, F. J., & Billings, S.A. (2009). The ecology of algal biodiesel production. Trends in Ecology and Evolution, 25(5), 301-309.

StockenreiteR, M., GrabeR, A.K., Haupt, F., & Stibor, H. (2012). The effect of species diversity on lipid production by micro-algal communities. Journal of Applied Phycology, 24, 45-54.

Striebel, M., Behl, S., Diehl, S., & Stibor, H. (2009). Spectral niche complementarity and carbon dynamics in pelagic ecosystems. American Naturalist, 174(1), 141-147.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., et al. (1997). The influence of functional diversity and composition on ecosystem processes. Science 277: 1300-1302.

Tilman, D., Reich, P.B., & Isbell, F. (2012). Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. Proceedings of the National Academy of Sciences, USA, 109(26), 10394-10397.

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T., et al. (2001). Diversity and productivity in a long-term grassland experiment. Science, 294, 843-845.

Wang, J., Zhang, C., Chen, T., & Li, W. H. (2013). From selection to complementarity: the shift along the abiotic stress gradient in a controlled biodiversity experiment. Oecologia, 171, 227-235.

Weis, J.J. (2016). Effect of phytoplankton richness on phytoplankton biomass is weak where the distribution of herbivores is patchy. PLoS ONE, 11(5), e0156057.

Weis, J.J., MadrigaL, D. S., & Cardinale, B. J.. (2008). Effects of algal diversity on the production of biomass in homogeneous and heterogeneous nutrient environments: a microcosm experiment. PLoS ONE, 3(7), e2825.

Wendling, M., Büchi, L., Amossé, C., Jeangros, B., Walter, A., et al. (2017). Species interactions leading to transgressive overyielding in cover crop mixtures. Agriculture, Ecosystems and Environment, 241, 88-99.

Willig, M.(201)1. Biodiversity and productivity. Science, 333(6050), 1709-1710.

Yuan, Z., Yu, K., Epstein, H., Stefanova, K., & Zhang, R. (2015). Plant species richness is not consistently associated with productivity in experimental subalpine meadow plant communities. Folia Geobotanica, 50, 207-217.