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Community efficiency during succession: a test of MacArthur's minimization principle in phytoplankton communities

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Abstract. Robert MacArthur's niche theory makes explicit predictions on how community function should change over time in a competitive community. A key prediction is that succession progressively minimizes the energy wasted by a community, but this minimization is a trade-off between energy losses from unutilised resources and costs of maintenance. By predicting how competition determines community efficiency over time MacArthur's theory may inform on the impacts of disturbance on community function and invasion risk. We provide a rare test of this theory using phytoplankton communities, and find that older communities wasted less energy than younger ones but that the reduction in energy wastage was not monotonic over time. While community structure followed consistent and clear trajectories, community function was more idiosyncratic among adjoining successional stages and driven by total community biomass rather than species composition. Our results suggest that subtle shifts in successional sequence can alter community efficiency and these effects determine community function independently of individual species membership. We conclude that, at least in phytoplankton communities, general trends in community function are predictable over time accordingly to MacArthur's theory. Tests of MacArthur's minimization principle across very different systems should be a priority given the potential of this theory to inform on the functional properties of communities.

Key words: compensation; competition; disturbance; geometric biology; metabolism; resistance; resource; species interactions; stability.

Introduction

Understanding how efficiently communities uptake and use resources, and sources of variation in this efficiency, is a ubiquitous goal in ecology. Community efficiency has been used to examine many concepts, including links between biodiversity and function (Chapin et al. 1997), the complexity of food webs (Lindeman 1942) and the stability of ecosystems (Odum 1985). One of the most obvious, yet little understood, ways in which community efficiency varies is during ecological succession (Odum 1969). From one perspective, succession per se is the "exchange of an excess available energy in the present for a future increase in biomass" (Margalef 1963), but the rates at which this exchange of energy occurs and how much energy is wasted might change during succession.

MacArthur (1969, 1970) formalised these ideas to determine how energy use should change over time in a competitive community. MacArthur and theoreticians since (Brew 1982, Chesson 1990, Gatto 1990, Loreau 2010) predicted that such a community, obeying a number of assumptions (described in detail by Loreau 2010), follows a Lyapunov function, i.e. a function that is always positive and whose time derivative is always negative, except at equilibrium where it is zero. A system obeying a Lyapunov function will show monotonic trajectories that converge to an equilibrium point where it is minimized. In MacArthur's framework the Lyapunov function represents the total amount of energy wasted by a community (community inefficiency, Q) expressed as a function of successional stage. Energy wastage, in its simplest form, can be calculated as the sum of two components:

$$Q = U + B,$$

where U is the amount of resources that go unutilised and B is the cost of maintenance given by the energy lost to metabolism and natural death per unit time (Gatto 1990). MacArthur's theory thus predicts that competition should minimize the total energy wastage of a community in a progressive fashion over time – that is

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maximise efficiency of energy use. Importantly this prediction is independent of the temporal scale considered as, by implying a Lyapunov function, MacArthur's theory implicitly predicts a monotonic trend towards the minimization of Q (Loreau 2010) and succession is a dynamic sequence valid on any timescale (Pickett et al. 2009).

If over time the two components of energy wastage (resource utilisation and maintenance costs) change in opposing directions, it might be challenging to predict how succession alters the functional properties of communities (Loreau 2010). In a young community, energy waste from unutilised resources might exceed the costs of maintenance, whereas an older community might better consume available resources but also experience higher costs of maintenance (Gatto 1990). Trade-offs between competitive ability and mortality determine species turnover and dominance (Chesson 2000), but it is not known if these trade-offs occur at the community-level and their consequences for community function have not been explored.

MacArthur's minimization principle makes explicit predictions on how competition should modify community energy use over time, but these predictions have remained largely untested (Ghedini et al. 2018*a*). MacArthur's predictions resonate with those of Lotka's (1922) maximum energy flux principle and Odum and Pinkerton's (1955) maximum power principle, which were tested by other authors (Cai et al. 2006, DeLong 2008). These principles share with MacArthur's theory the prediction that some ecosystem-level property is maximised during the self-organization of communities, but they differ in that it is neither total energy flux nor power output that are maximized under MacArthur's theory. MacArthur's minimization principle predicts that communities maximise the efficiency of energy use, and has the advantage of combining both resource use and maintenance costs – this combination means that a competitive community might result from the trade-off between two quantities changing in opposing directions.

Resolving whether succession maximises efficiency is an important first step to predict the effects of anthropogenic disturbance on energy flows. Human activities can disrupt successional and competitive dynamics by altering resource availability (nutrient enrichment) or reducing the size of organisms (exploitation, warming) (Daufresne et al. 2009, Chang and Turner 2019). These disturbances could maintain communities closer to their early successional stages which might be the ones with faster biomass production, but not necessarily the most efficient in terms of function (e.g. O_2 production, C storage, nutrient cycling) (Odum 1969). While compensatory dynamics among species can stabilise community processes (McNaughton 1977, Gonzalez and Loreau 2009), the directional nature of many disturbances could pose greater strain on such responses to maintain function (Loreau 2010).

If MacArthur's theory is correct, it may also inform on the stability and invasion risk of communities beyond the effects of biodiversity. An efficient community might be more resistant to invasion because fewer resources are available for an invading species (Levine et al. 2004). While biodiversity increases efficiency of resource use (Cardinale et al. 2012), more diverse communities are not always less invaded (Fridley et al. 2007). Specific species traits might drive community resource use independently of diversity (Hodapp et al. 2019). For instance, older communities, such as well-established forests, might have relatively low diversity but might nonetheless be very efficient at utilising resources – possibly because succession maximises efficiency, as MacArthur predicted.

The ecological importance of phytoplankton communities cannot be underestimated as phytoplankton forms the basis of marine food webs and contributes nearly half of global primary production and carbon fixation (Field et al. 1998). Environmental changes are altering the composition and size structure of these communities, creating uncertainty about their future function (Padfield et al. 2018). Understanding how the efficiency of phytoplankton communities varies with species turnover is therefore essential to predict how climate change will influence global carbon cycles.

We use phytoplankton communities to test MacArthur's model predictions that (1) overall energy wastage (Q) is minimized during succession, and (2) its two components change in opposing ways, i.e. unutilised resources decrease over time (U), whereas energy lost to maintenance increases (B). To quantify changes in energy wastage during succession we measured changes in resource use (light capture, gross productivity, nutrient uptake) and maintenance costs (metabolism, mortality) at weekly intervals. We then calculated resource waste (U) as the sum of two components, i.e. light not captured and light captured but not used for production. Similarly, maintenance (B) was calculated as the sum of metabolic costs and natural mortality as defined in MacArthur's framework (Chesson 1990, Gatto 1990, Loreau 2010). Concomitantly, we quantified changes in biovolume, abundance and size for the whole community and individual species. This experiment expands our previous test of MacArthur's theory on heterotrophic communities of marine invertebrates (Ghedini et al. 2018*a*) on a completely different system. The new phytoplankton system meets a key assumption that was violated in our previous test of the theory: competitive communities are a closed system where competition determines community composition (i.e. which species survive). Ghedini et al. (2018*a*) used a field model system where sessile invertebrate communities were open to immigration and exposed to natural resource inputs and predation. In the current experiment we utilise a microcosm phytoplankton system where dynamics of energy use are solely determined by a closed set of competing species receiving fixed resource input and not exposed to predation (see Appendix S1 for a description of how the model system

meets the assumptions of MacArthur's theory). By moving from a heterotrophic, multicellular system to an autotrophic unicellular system, the results presented here inform on whether temporal changes in community function are predictable across very different communities.

Materials and Methods

Experimental communities

We experimentally created communities of marine phytoplankton by mixing equal biovolumes of six species (*Amphidinium carterae*, *Amphora coffeaformis*, *Tetraselmis* sp., *Dunaliella tetriolecta*, *Synechococcus* sp. and *Tisochrysis lutea*). These cosmopolitan species are found in coastal areas globally and were chosen for their capacity to grow well in laboratory conditions and to represent different algal groups and cell sizes ranging from 4 to ~600 μm^3 (Appendix S2: Table S1). Species were sourced from the Australian National Algae Culture Collection and reared as individual populations for two months. On the day of experimental set-up, we determined the biovolume ($\mu m^3/\mu L$) of each species based on their cell density (cells/μL) and cell volume (μm^3). Cell densities were determined from photos taken with an Olympus light microscope at 400× using a Neubauer haemocytometer from 2 × 10 μL lugol-stained samples. Cell size was measured from photos taken from a 10 μL sample on 50 cells per species using ImageJ and Fiji (version 2.0; Schindelin et al. 2012). Cell volume was calculated by assigning the approximate geometric shape to each species (Hillebrand et al. 1999; Appendix S2: Table S1). We chose a target biovolume equal among all species (355×10^7 μm^3 for run 1 and 176×10^7 μm^3 for run 2 – this difference was due to differences in the cell density of species cultures between runs) such that their combined volume was approximately 1/5 of the total volume (100 mL out of 500 mL) and filled the remaining with medium. We used the standard enriched seawater medium designed for growing coastal marine algae prepared with 0.45 μm filtered seawater and containing 8.82×10^{-4} mol/L of nitrogen and 3.62×10^{-5} mol/L of phosphorous (f/2 medium with silica, Guillard 1975).

We set-up a total of 20 communities split in two runs of 10 replicate communities each. The two runs were analysed independently as they were initiated four weeks apart. Starting in September 2018, successional changes in each community were sampled weekly from one day after set up (week 0) for ten weeks (week 9). Since phytoplankton division rates are of the order of hours to few days (~1 d, Banse 1991), ten weeks would allow us to observe a number of successional changes. Communities were grown using a semi-continuous culture technique in clear-glass flow-through vessels (chemostats) of 500 mL volume in a temperature-controlled laboratory at 22 ± 1°C. When necessary the sides of the chemostats were cleaned with sterile cannulas. This approach was preferred to a batch transfer to avoid introducing a disturbance at every transfer, potentially disrupting the successional process. Air constantly bubbled from the bottom of chemostats to ensure mixing and carbon availability. Nutrients were added twice daily (every 12 h) as two inputs of 50 mL of the standard f/2 medium described above *via* a peristaltic pump (Kamoer X4 Dosing Pump, Kamoer Fluid Tech Co., Ltd., Shanghai, China) with a dilution rate of 0.2 d^{-1}. An overflow outlet maintained a constant volume and the overflow was collected in a container and used for sampling (see next section). When required, salinity was adjusted with few drops of distilled water. Cool white fluorescent lamps provided light on a 14–10 h day-night cycle set at two non-saturating irradiance levels (Six et al. 2004, Edwards et al. 2016): 75.4 ± 3.9 μmol photons·m^{-2}·s^{-1} for run 1 and 111.2 ± 5.4 for run 2.

Community structure

On each sampling day, cell density (cells/μL) and cell volume (μm^3) of each species were determined using the methods described above. Cell size was measured on 20 cells per species in each community – in some cases <20 cells or no cells were present as some species became extremely rare or went extinct. Total community biovolume was calculated as the sum of individual species biovolumes ($\mu m^3/\mu L$).

Energy losses from light

Each week we measured the amount of light not captured by each community by measuring the photosynthetically active radiation passing through the 50 mL outflow of each community (μmol photons·s^{-1}·m^{-2}) with a light meter (Quantum Meter, Apogee Instruments Inc., Utah USA). At the last sampling time we also measured the light passing through the whole community at increments of 50 mL to determine the relationship between light lost by 50 mL (x) and light lost by the whole community (y): $y = 0.19 \cdot x - 2.74$, $R^2 = 0.49$ for run 1, and $y = 0.20 \cdot x - 5.89$, $R^2 = 0.41$ for run 2. We then used these relationships to calculate light losses for the whole community at previous sampling times based on measurements of light lost by 50 mL.

The amount of light not captured represented the first component of resource wastage (U1) and was converted from μmol photons·s^{-1}·m^{-2} to energy (J·s^{-1}·m^{-2}) using the conversion factor 0.327 Joules/μmol for sunlight radiation assuming that our fluorescent lights provided a similar spectrum (www.apogeeinstruments.com/conversion-ppfd-to-watts). Daily energy losses from light not captured and were calculated over 14 h of light for the horizontal section (19.6 cm^2) of the chemostats (J/d). These energy losses were used to calculate the energy absorbed from light as the difference between the energy that each chemostat received from light and the light not captured. The energy absorbed from light will then be

used to calculate the second component of resource wastage, i.e. light absorbed but not used for production (U2, described in "*Statistical analyses and estimates of energy waste*").

Photosynthesis and metabolism

Rates of photosynthesis and respiration were measured on five replicate samples (in 5 mL vials) from each community and calculated from change in percentage oxygen saturation using 24-channel PreSens sensor dish readers (SDR; AS-1 Scientific, Wellington, New Zealand) following protocols of Malerba et al. (2017). Briefly, sensors were calibrated with 0% and 100% air saturation before the experiment. Samples were placed on the side under the light source to avoid cell deposition on the oxygen sensor and sodium bicarbonate (2 mmol/L) was added to ensure photosynthesis was not limited by carbon availability. Ten blanks were filled with the supernatant obtained by centrifuging 14 mL of each community and used as controls to correct for background microbial activity. Vials were fully filled, being careful to remove all air pockets. Oxygen production was measured under six light intensities (50–300 μmol photons·m^{-2}·s^{-1} at increments of 50), randomizing the order of light intensities at each sampling time. Each light intensity was followed by a 30-min dark period to measure metabolic costs. At each light intensity, the rate of oxygen production or consumption of the whole sample (VO_2; units μmol O_2/min) was measured following White et al. (2011):

$$VO_2 = 1 \times ((m_a - m_b)/100) \times V\beta O_2,$$

where m_a is the rate of change of O_2 saturation in each sample (min^{-1}), m_b is the mean O_2 saturation across all blanks (min^{-1}), V is the water volume (0.005 L), and βO_2 is the oxygen capacity of air-saturated seawater at 20°C and 35 ppt salinity (225 μmol O_2/L).

The average rate of oxygen production or consumption (μmol O_2/min) from the five replicate vials was converted to calorific energy (Joules/min) using the conversion factor of 512×10^{-3} Joules/(μmol O^2) (Williams and Laurens 2010) and divided by the volume of the vial (5 mL) to obtain rates per mL (J·min^{-1}·mL^{-1}). Oxygen consumption was calculated separately for the first 15 min of dark as this faster oxygen consumption indicated enhanced-post illumination metabolic rates (Beardall et al. 1994). The following 15 min of respiration showed a shallower decline and were taken as a measure of the slower dark metabolism. As it is unknown whether enhanced-post illumination rates occur throughout daytime (Beardall et al. 1994, Griffin and Turnbull 2012), to be conservative in our estimates of metabolic costs we calculated metabolism as the average of hourly rates of light and dark metabolism. We used this average metabolic rate to calculate daily gross photosynthesis (photosynthesis plus metabolism) over 14 h of light, and daily metabolic costs over 24 h (J/mL).

Mortality

Cell mortality was estimated by adding 10 μL of DAPI stain (diamidino-2-phenylindole, 1 μg/mL) to a 100 μL sample of each community. DAPI is blue fluorescent probe that binds to DNA content penetrating the cellular membrane of dead cells (Porter and Feig 1980, Gallardo-Escarate et al. 2007). Cells stained with DAPI were incubated in the dark for 15 min prior to taking photos of a 10 μL sample under a fluorescent inverted miscroscope (Leica DMi8) using the DAPI channel (excitation = 325–375 nm; emission = 435–485 nm). For each species, mortality was determined as percentage of dead cells and converted to percentage dead biovolume to calculate the total dead biovolume (μm^3) for that species in the community. Dead biovolume was converted to energy based on species-specific carbon content per unit volume (pc C/μm^3) (Appendix S2: Table S1; Verity et al. 1992, Menden-Deuer and Lessard 2000). Carbon content was converted to energy assuming that 1 mg of carbon equals 47.7 Joules (Platt and Irwin 1973). The contribution of each species was summed to obtain the total energy lost to mortality by the whole community. We divided our estimates by six to obtain rates of daily mortality, as trials showed that cell decomposition occurs over a timespan of six days.

Nutrients uptake

The algal pellet obtained from each community to make up oxygen blanks was resuspended in 200 mL of fresh medium (standard f/2 medium described above) to determine the uptake of nitrogen (NO_x-N) and phosphorous (PO_4-P) at weeks 0, 5 and 9. To quantify nutrient uptake we collected one 15 mL sample from each resuspended community (n = 10) after six hours. Control samples were also collected at this time from three control vials filled only with media to quantify nutrient availability in the seawater medium in the absence of consumers. Samples were filtered through a 45 μm syringe filter and frozen at −20°C until analysis (Quickchem Flow Injection Analyser; Lachat Instruments, Loveland, Colorado, USA). Nutrient uptake (mg/L) was determined as the average amount of nutrients present after six hours in the controls minus that in each community, and converted to percentage.

Statistical analyses and estimates of energy waste

The data from the two runs were analysed separately. Linear mixed models were used to test how community structural properties (total biovolume, total cell abundance and average cell size), resource use (light not captured, gross photosynthesis and nutrient uptake) and

maintenance costs (mortality and metabolism) changed over time. For these analyses we used metabolism and gross photosynthesis rates (J/d) measured at a light intensity of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. We included time as a fixed categorical effect (the repeated measure, 10 sampling times) and community (n = 10) as a random effect with random intercept but fixed slopes. Data of biovolume, size and abundance were $\log_{10}$-transformed prior to analyses.

Resource waste (U) was calculated as the sum of two components: the daily energy lost as light not captured (U1) and the daily energy wasted as light captured but not utilised for production (U2). The second component (U2) was the difference between the energy absorbed from light (energy provided by light minus light not captured) and the energy produced by gross photosynthesis. In a perfectly efficient system, this difference would be zero. However, light backscattering and intracellular self-shading mean that not all incident photons are captured by the light harvesting system and channelled into gross photosynthesis (Malerba et al. 2018*b*). Gross photosynthesis rates were estimated specifically for the average light intensity of each run using parameter estimates obtained from mixed effects models for the effects of successional stage (time) and light intensity (50–300 μmol photons$\cdot m^{-2} \cdot s^{-1}$) on gross photosynthesis (J/mL), and then multiplied by 500 mL to calculate values for the whole community. "Community" was included as random factor allowing the intercept to vary but keeping constant slopes across time.

The daily cost of maintenance (B) was calculated as the sum of two components: the energy lost to mortality (B1, estimated from repeated measure analysis described above) and the energy lost to metabolism over 24 h (B2) estimated from the metabolic rate (J/mL) in the same way as gross photosynthesis, and calculated for the whole community (500 mL). Finally, total energy wastage per day (Q) was calculated as the sum of U and B. While MacArthur's predictions refer to total community energy wastage, we also standardised Q by total community biovolume to assess temporal fluctuations in energy use per unit volume ($J \cdot \mu m^{-3} \cdot \mu L^{-1}$). Statistics were done in R version 3.6.1 using packages *lme4* (Pinheiro et al. 2016) and *lsmeans* (Lenth 2016).

Results

Community biovolume and composition

Successional changes in community structure were similar between the two runs (Fig. 1). Total biovolume increased but not in a linear manner. It was lowest initially and, after increasing rapidly in the first two weeks, it fluctuated to a lesser degree throughout succession peaking before the final stages ($F_{9,81}$ = 10.03, P < 0.001; week 3 = 7 > 9 > 0, Fig. 1a for run 1; $F_{9,81}$ = 25.77, P < 0.001; week 5 = 8 = 9 > 0, Fig. 1d for run 2). Total cell abundance increased rapidly ($F_{9,81}$ = 35.01, P < 0.001, week 1 = 2 > 0 > 9, Fig. 1b for run 1; $F_{9,81}$ = 18.84, P < 0.001, week 2 = 1 > 9 = 0, Fig. 1e for run 2) because of an initial increase in small cells (*Synechococcus*; Appendix S2: Fig. S1a). Over time abundance declined as larger species (*Amphidinium* and *Tetraselmis*, but not the diatom *Amphora*) dominated biovolume (Appendix S2: Fig. S1b). Changes in species dominance led to an initial decline in size followed by a progressive increase in average cell size over time ($F_{9,81}$ = 57.04, P < 0.001, Fig. 1c for run 1; $F_{9,81}$ = 28.99, P < 0.001, Fig. 1f for run 2). Within species there was a tendency for a decline in size over time but relative differences among species remained similar (Appendix S2: Fig. S1c). While changes in community structure were similar between the two runs, dynamics of energy use exhibited slightly different temporal patterns so we report them separately.

Run 1 – Resource use: light, photosynthesis and nutrients.—The amount of light not captured (U1) initially declined (weeks 1–3) but then increased such that older communities lost similar amounts of light as initial communities ($F_{9,81}$ = 6.12, P < 0.001, week 0 = 9 > 1 = 3, Appendix S2: Fig. S2a). The energy lost as light not used for production (U2) declined over time but in a non-monotonic way reaching the lowest values at intermediate stages ($F_{9,81}$ = 923.6, P < 0.001, week 0 > 9 > 7 = 3 = 4, Appendix S2: Fig. S2b).

Gross productivity increased over time but fluctuated among weeks such that initial communities were the least productive, mid-stages were the most productive and final communities were intermediate ($F_{9,80}$ = 13.2, P < 0.001, week 3 > 9 > 0, Appendix S2: Fig. S3a). Communities consumed similar amounts of nitrogen as they grew older, but mid-stages and to a lesser extent late-stages consumed more phosphorous than the initial communities (time × nutrient: $F_{2,45}$, P = 0.057; Appendix S2: Fig. S3b).

Run 1 – Energy wastage.—Resource waste (U) was determined by the sum of the energy wasted as light not captured (U1) and light captured but not used for production (U2) (Appendix S2: Fig. S2). Resource waste declined over time but in non-monotonic ways: it was highest for initial communities, lowest for mid-stages (weeks 3, 4 and 7) and intermediate for final communities (Fig. 2a). The temporal pattern of resource waste was mostly driven by light captured but not used for production (Fig. 2b).

Costs of mortality were low and constant over time ($F_{9,90}$ = 0.99, P = 0.46, Appendix S2: Fig. S4a). Conversely, the cost of metabolism increased from early to late stages in a non-monotonic manner following changes in photosynthesis, thus peaking at week 3 and 7 and taking intermediate values for final communities ($F_{9,81}$ = 11.75, P < 0.001, week

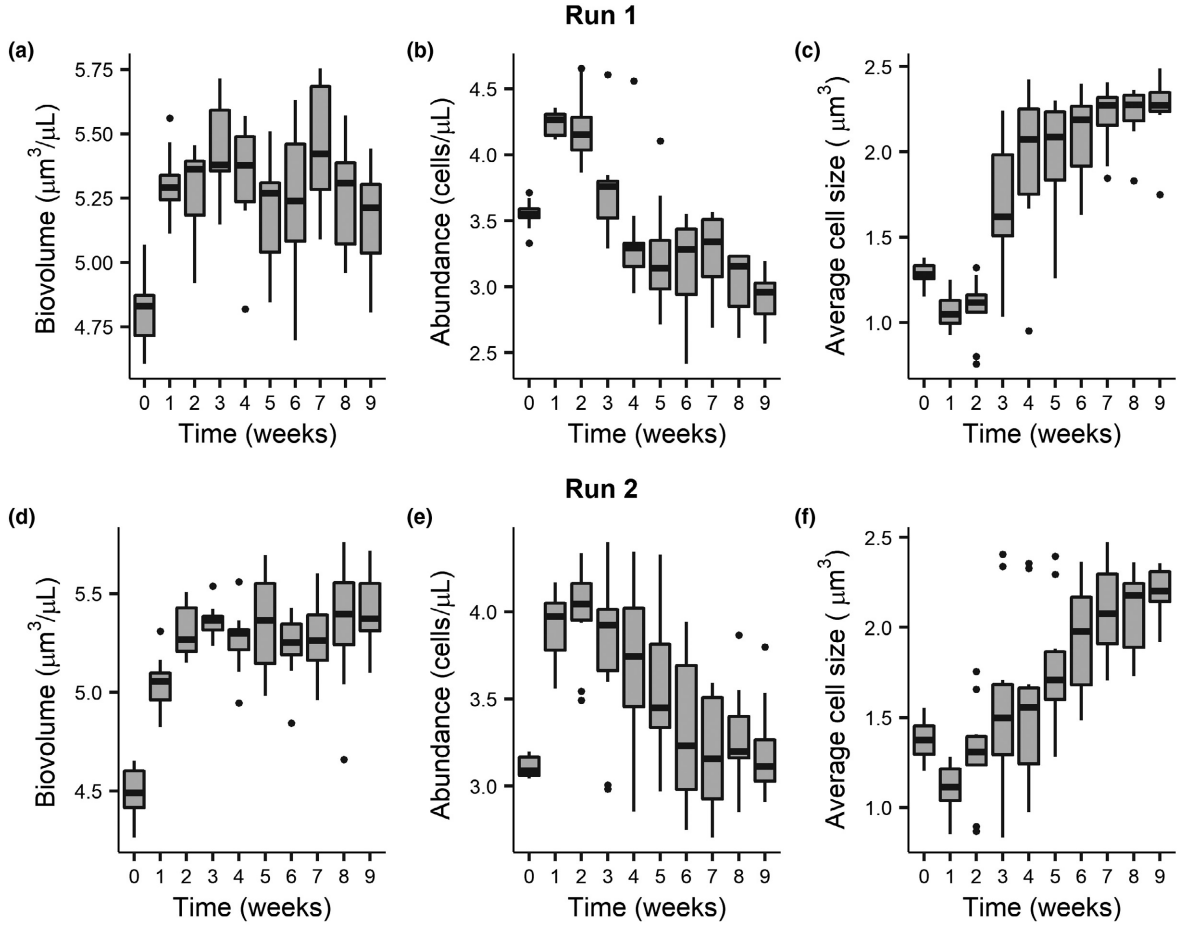

FIG. 1. Changes in total biovolume (a, d), total cell abundance (b, e) and average cell size (c, f) over time for run 1 (top) and run 2 (bottom). All data are $\log_{10}$-transformed.

3 = 7 > 9> 0; Appendix S2: Fig. S4b). Overall maintenance costs (*B*) were driven by metabolism and were very low for initial communities, increasing over time but in a non-monotonic manner and surpassing resource waste only for intermediate communities (week 3, 4 and 7) (Fig. 2a).

Total energy waste (*Q*) declined over time but with a non-monotonic trend determined by the opposing changes in resource waste and maintenance costs (Fig. 2b). Initial communities sustained the highest total energy losses because, even if they had the lowest metabolic costs, they wasted most resources. Mid-stages (weeks 3, 4 and 7) had the lowest energy waste because, while they had the highest metabolic costs, they wasted the least resources. In comparison, the efficiency of older communities was intermediate because they wasted more resources but had lower metabolic costs than the most productive mid-stages. Fluctuations in energy wastage were mostly explained by changes in total community biovolume because energy waste per unit biovolume was similar across all successional stages, with the exception of the intial communities which had higher waste per unit biovolume (Fig. 2c).

Run 2 – Resource use: light, photosynthesis and nutrients.—The amount of light not captured (U1) followed a different trajectory than that observed in Run 1 as losses progressively declined over time ($F_{9,81}$ = 10.47, P < 0.001, week 0 > 3 > 9, Appendix S2: Fig. S2c). The energy lost as light not used for production (U2) declined over time in a non-monotonic way but in a more progressive fashion than that observed in Run 1 ($F_{9,81}$ = 707, P < 0.001, week 0 > 9 > 8, Appendix S2: Fig. S2d).

Gross productivity followed a similar pattern of change to that of Run 1, increasing over time in a non-monotonic way, with the difference that final communities were on average the most productive ($F_{9,81}$ = 14.08, P < 0.001; week 9 > 6 > 0; Appendix S2: Fig. S3c). Nutrient consumption remained similar over time, although later stages tended to consume more phosphorous (nutrients × sampling: $F_{2,45}$, P = 0.7; Appendix S2: Fig. S3b).

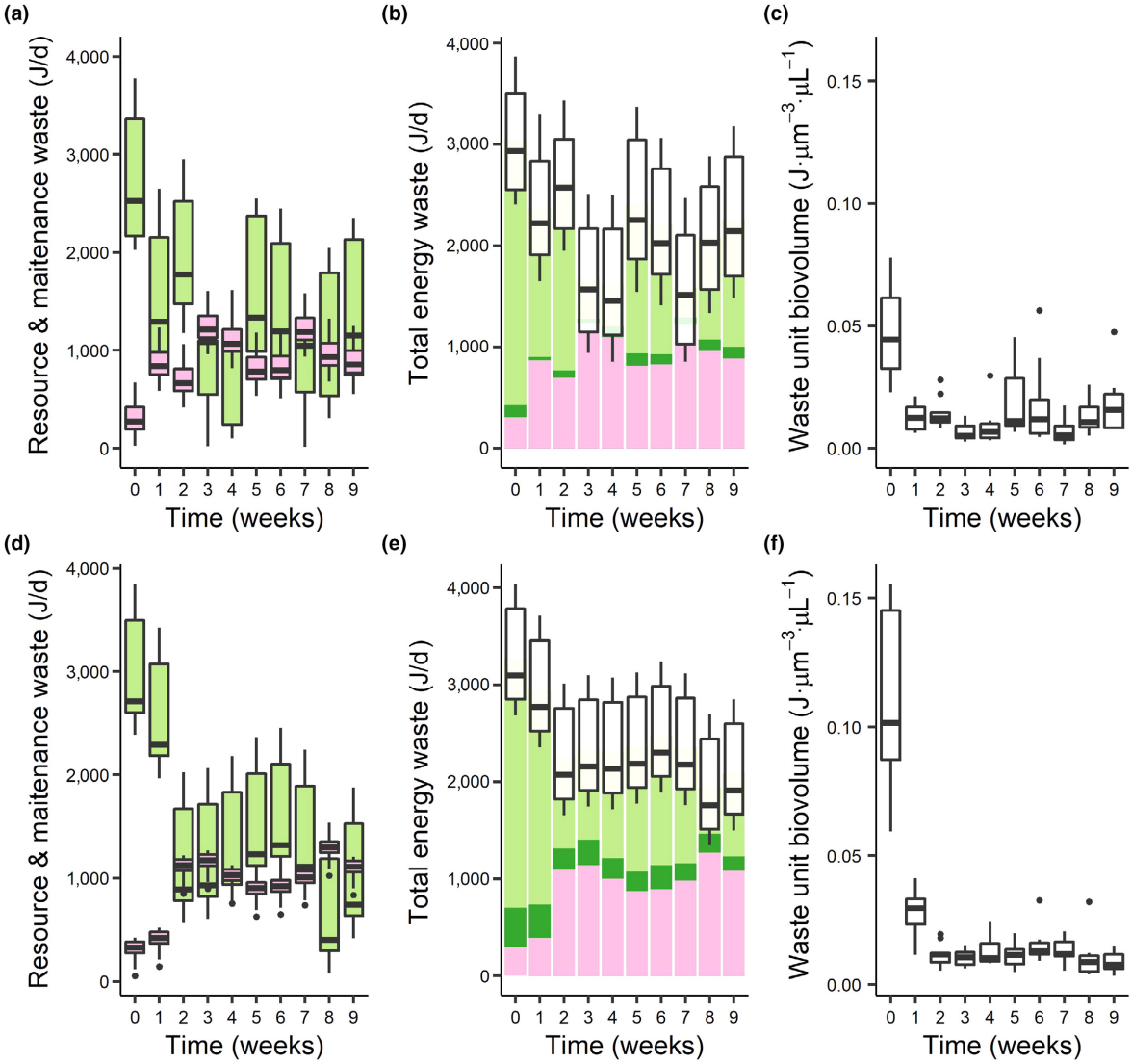

FIG. 2. Changes in community energy waste during succession for run 1 (top) and run 2 (bottom). The energy lost from unutilised resources (*U*, green), and maintenance (*B*, magenta) change in opposing ways over time (a, d). Total energy wastage (*Q*, white boxplot) declines but in non-monotonic ways because of changes in the relative contribution of *U* and *B* at each successional stage (b, e). Each component of energy wastage has a different color (light green = light captured but not used for production, dark green = light not captured, magenta = metabolic costs). Total energy wastage (*Q*) per unit of total biovolume declines rapidly in the first week but then remains similar over time (c and f).

Run 2 – Energy wastage.—Resource waste (*U*), given by the combination of light not captured (U1) and light not used for production (U2) (Appendix S2: Fig. S2), decreased over time in a more progressive way than Run 1, as it was highest for initial communities, lowest at week 8 and slightly higher for final communities (Fig. 2d). Resource waste was again mostly determined by the energy lost as light not used for production (Fig. 2e).

Costs of mortality were low throughout succession and, even if mortality at week 5 was greater than that at time 0 ($F_{9,81}$ = 2.13, P < 0.05, Appendix S2: Fig. S4c), there was no difference among all other sampling times. Metabolic costs increased from early to late stages in a non-monotonic manner following changes in photosynthesis, and were lowest for initial communities and highest for the oldest communities ($F_{9,81}$ = 18.49, P < 0.001, Appendix S2: Fig. S4d). As for run 1, maintenance costs (*B*) were driven by metabolic costs and increased over time peaking at week 8 but remaining similarly high for final communities (Fig. 2d). The cost of maintenance exceeded resource wastage for the final two stages (Fig. 2d).

Total energy waste (*Q*) declined over time following opposing changes in resource waste and maintenance costs (Fig. 2e). As in run 1, initial communities sustained the highest energy losses having low maintenance costs and high resource waste. Older communities (the final

two stages), rather than mid stages, had the lowest total waste across succession as they had higher maintenance but lowest resource wastage. Similarly to run 1, total energy waste per unit biovolume declined sharply initially (week 0–1) and then remained relatively constant throughout succession (Fig. 2f).

Discussion

Overall we found support for MacArthur's predictions that during succession (1) total energy waste declines and (2) waste from unutilised resources and maintenance costs change in opposite directions (Table 1). The opposing changes in unutilised resources and maintenance costs can be best visualised by the negative relationship between the declining energy not used for production and increasing metabolic costs. Although later stages were better at exploiting resources (reducing the *U* component), these gains were partially offset by increasing metabolic costs to sustain production (*B* component). Thus, by meeting MacArthur's assumptions of a system closed to immigration and predation, this new study validates MacArthur's predictions that were only partially verified in our previous experiment (Ghedini et al. 2018*a*).

While our results are qualitatively similar to MacArthur's, we did not observe monotonic reductions in energy wastage over time. We also observed that, while metabolic costs increased, older communities did not have the highest metabolic rates or energy flux as predicted by Lotka (1922) maximum energy flux principle. Fluctuations in energy waste among successional stages meant that older communities were not always the most efficient, a result that echoes our previous finding (Ghedini et al. 2018*a*). MacArthur's model predicts a monotonic increase in efficiency, but this property might not hold for ecological systems that show transient dynamics in efficiency over time. In a similar way, ecological systems often show transient "stable" stages during succession towards a climax community (Glenn-Lewin et al. 1992), with peaks in production at intermediate stages (Lindeman 1942). Although community function had an idiosyncratic trajectory, phytoplankton communities followed general patterns of succession with production, respiration and biomass increasing over time (Odum 1969), and structural properties (species abundance, size and dominance) showing clear and consistent patterns leading to the dominance of two species. Hence, competition can lead to complex trajectories of energy use even when there are clear patterns of species dominance. MacArthur originally assumed that resources had faster dynamics than their consumers, whereas in our system nutrients were added daily but could still become limited within that day. Nonetheless, if the superior competitors maximise resource use, community efficiency should still be maximised (Gatto 1990, Loreau 2010). Regardless, our results offer qualified support for MacArthur's minimization principle in phytoplankton communities.

Could the homogenization of communities (loss of diversity) explain why older communities were not always the most efficient? Trait homogenization can reduce community function by decreasing resource use efficiency (Cardinale et al. 2011). Our initial communities were however the most inefficient despite being the most diverse and even (initial evenness = 0.97 versus final = 0.32 for run 1; 0.94 versus 0.29 for run 2). While many community properties changed during succession, total community biovolume alone explained most of the variation in energy wastage among successional stages.

It is remarkable that increases in average cell size over time did not affect the efficiency of communities per unit biovolume. The average cell size increased from ~20 to 170 μm^3 over the course of the experiment, but energy flux per unit of total biovolume remained relatively stable. Increases in average size would be predicted to alter community energy flux given that larger organisms have lower mass-specific metabolic rates than smaller organisms (Brown et al. 2004). Accordingly, some studies found that community energy fluxes depend on size structure (Yvon-Durocher and Allen 2012) and succession increases efficiency per unit mass (Boit and Gaedke 2014). Similarly, based on increases in cells size, we would have predicted a decrease in energy use per unit biovolume of the community. Instead, these fluxes were consistent in time despite massive increases in size. Other studies have found that community rates can be size-independent (Huete-Ortega et al. 2011, Padfield et al. 2018, Ghedini et al. 2018*b*). Together these results suggest that aggregate community properties can be more stable over time than the underlying dynamics of species traits. Whilst succession drives structural changes, their effects on functional properties might be dampened with increasing scale of complexity (Ruesink and Srivastava 2001, Gonzalez and Loreau 2009, Ghedini et al. 2015). The dissimilarities among trajectories of community function, however, highlight that the extent to which species traits drive higher-level processes is not well understood, and simply scaling up from species to communities is fraught even when it comes to fundamental physiological processes.

Table 1. Summary table of results.

Source of energy waste	Predicted direction of change	Observed	
		Run 1	Run 2
Light not captured (U1)	Decrease	No	Yes
Light not used (U2)	Decrease	Yes	Yes
Resource waste (U)	Decrease	Yes	Yes
Mortality (B1)	Increase	No	No
Metabolism (B2)	Increase	Yes	Yes
Maintenance (B)	Increase	Yes	Yes
Total energy waste (Q)	Decrease	Yes	Yes

Our results are qualitatively similar to MacArthur's predictions, with the exception of light not captured in run 1 and mortality in both runs, but none of the observed responses changed monotonically over time.

Our finding that larger species tend to dominate over time confirms the proposed advantages of increased cell size in phytoplankton, which may explain the paradoxical trajectories of resource use in our communities. Populations of larger species tend to grow at a slower pace (Marbà et al. 2007) and have a lower net-energy production per unit volume than smaller cells (Malerba et al. 2017), but they capture and store resources more efficiently sustaining greater total biomass (Marañón 2015, Malerba et al. 2018*a*). The later successional dominance of large cells could thus be a function of the intermittent nutrient supply, which favours K-strategists over r-strategists (Papanikolopoulou et al. 2018), or of mean nutrient availability – a lower nutrient input could favour smaller cells which usually dominate in oligotrophic conditions (Marañón 2015). Larger cells have lower nitrogen requirements per unit volume than smaller cells (Marañón et al. 2013), potentially explaining why older communities showed similar amount of nitrogen uptake relative to initial communities despite their much greater total biovolume. However, since our experiment included few species per size class we can only hypothesise that dominance is driven by size rather than some other trait.

Global change can disrupt successional dynamics by altering competition between species with different trade-offs of resource uptake and metabolic costs (Marañón 2015). One of the dominant trends observed in phytoplankton is a shift towards smaller sizes under warming (Daufresne et al. 2009). Phytoplankton productivity can be negatively impacted by lower trait diversity but these effects seem contingent on environmental conditions (Vallina et al. 2017). We found that neither changes in dominant species nor the homogenisation of communities had clear effects on energy flux. Nonetheless, small-sized species tend to be more common in young communities, which we found were overall less efficient than older communities. Communities dominated by small species might also be more susceptible to nutrient-depleted conditions (Malerba et al. 2018*a*). Combined reductions in size and resource availability projected under warming may reduce the strength and efficiency of the carbon pump, as well as the amount of standing biomass at the basis of marine food webs.

In conclusion, older communities wasted less energy than younger communities but this decline was not monotonic and later stages were not consistently the most efficient, indicating that competition does not lead to linear changes in community energy use. Total community biovolume rather than species composition or size was the main driver of changes in efficiency, suggesting a greater stability of community functional properties than what would be predicted based on individual species membership. The results from these experiments and our previous work (Ghedini et al. 2018*a*) indicate that overall trajectories in community function may be predictable over time, albeit with fluctuations at smaller scales, and that MacArthur's minimization principle might apply across very different systems.

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Supporting Information

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Data Availability

Data are available on Figshare: https://doi.org/10.26180/5e2a1a8d74be7.