LETTER

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Biodiversity improves water quality through niche partitioning

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Excessive nutrient loading of water bodies is a leading cause of water pollution worldwide[1,2], and controlling nutrient levels in watersheds is a primary objective of most environmental policy[3]. Over the past two decades, much research has shown that ecosystems with more species are more efficient at removing nutrients from soil and water than are ecosystems with fewer species[4–7]. This has led some to suggest that conservation of biodiversity might be a useful tool for managing nutrient uptake and storage[7–10], but this suggestion has been controversial, in part because the specific biological mechanisms by which species diversity influences nutrient uptake have not been identified[10–12]. Here I use a model system of stream biofilms to show that niche partitioning among species of algae can increase the uptake and storage of nitrate, a nutrient pollutant of global concern. I manipulated the number of species of algae growing in the biofilms of 150 stream mesocosms that had been set up to mimic the variety of flow habitats and disturbance regimes that are typical of natural streams. Nitrogen uptake rates, as measured by using ^{15}N-labelled nitrate, increased linearly with species richness and were driven by niche differences among species. As different forms of algae came to dominate each unique habitat in a stream, the more diverse communities achieved a higher biomass and greater ^{15}N uptake. When these niche opportunities were experimentally removed by making all of the habitats in a stream uniform, diversity did not influence nitrogen uptake, and biofilms collapsed to a single dominant species. These results provide direct evidence that communities with more species take greater advantage of the niche opportunities in an environment, and this allows diverse systems to capture a greater proportion of biologically available resources such as nitrogen. One implication is that biodiversity may help to buffer natural ecosystems against the ecological impacts of nutrient pollution.

Over the past century, humans have more than doubled the rate of nitrogen input into terrestrial ecosystems, mostly through fossil fuel combustion and increased use of agricultural fertilizers[1,2]. Excess nitrogen flows into streams and rivers, where it contributes to eutrophication, one of the leading causes of degraded water quality worldwide[13,14]. If it is not removed by biotic uptake or denitrification, nitrogen is ultimately exported from rivers to estuaries and coastal oceans[15,16], where it can promote blooms of harmful microorganisms[17] and can generate an excessive biochemical oxygen demand, which has resulted in 245,000 km^2 of 'dead zones' in more than 400 coastal habitats around the world[18]. Given the global ecological and economic impact of these environmental problems[19], a primary objective of environmental policy and management is to control the input of nitrogen into watersheds and to maximize its removal[3].

A growing body of recent research has suggested that conservation of biodiversity might be a useful management tool for reducing the concentrations of nitrogen in soil and water[4–10]. It is often argued that communities with more species take greater advantage of the niche opportunities that are available in an environment than do those with fewer species, and this allows diverse ecosystems to capture a greater proportion of biologically active resources such as nitrogen[20,21]. But the role of niche differences among species in regulating nutrient uptake has rarely been measured directly[6,12,22]. Instead, effects of niche differences on nutrient uptake either have been assumed to occur based on theoretical arguments[20,23] or have been inferred from post hoc statistical analyses of experiments that have been unable to differentiate among specific biological mechanisms[24]. Because of the lack of direct evidence confirming a biological mechanism, there has been considerable debate about why diverse ecosystems tend to be more efficient at sequestering dissolved nutrients[10–12].

Here I present the results of a novel experiment in which I directly manipulated algae and their niche opportunities in a large set of stream mesocosms to examine the mechanistic links between the diversity of algae, niche differences among species and the rate of nitrate removal from stream water. I isolated cultures of eight of the most widespread species of diatoms and green algae that inhabit streams in North America: five species of Bacillariophyceae (the diatoms *Achnanthidium minutissimum*, *Melosira varians*, *Navicula cryptocephala*, *Nitzschia palea* and *Synedra ulna*), and three chlorophyceaen green algae (*Scenedesmus quadricauda*, *Stigeoclonium* sp. and *Spirogyra* sp.) (Supplementary Tables 1 and 2). These species were then used to colonize recirculating stream channels with equal initial cell densities of one, two, four, six or eight species (see Methods). Streams were set up to mimic two forms of environmental heterogeneity that are typical of natural fluvial ecosystems and that are thought to provide niche opportunities allowing species to coexist. Flow heterogeneity was created by arranging algal growth surfaces so that velocities near the stream bed varied from <2 to 55 cm s^{-1}. The abundance, distribution and diversity of stream algae are known to be influenced by a stream's flow regime[25], and species have evolved varying morphological adaptations to cope with near-bed shear stress[26,27]. In addition to spatial heterogeneity in flow, temporal heterogeneity was created by dividing the growth surfaces in a stream into 18 distinct habitat patches (2.5×2.5 cm^2), each of which had a probability of 0.25 of being disturbed (by removal with a brush) in each week of the 6-week experiment. This treatment produced a successional mosaic of patches that varied from 5 to 50 days old by the end of the experiment. Different species often dominate habitats at different stages of succession owing to trade-offs between the ability of species to colonize the available space versus their ability to compete with other species. These trade-offs can enhance coexistence in streams that experience periodic disturbances[28].

In heterogeneous streams, nitrogen uptake rates were a linear function of species diversity across the range of richness used in this study, increasing by 0.06 μg NO_3^- cm^{-2} h^{-1} for each species of alga in the biofilm (Fig. 1a and Table 1). The most diverse algal polyculture (eight species) removed nitrate 4.5-fold faster than the average rate of a species grown alone (in monoculture). Rates of uptake in streams colonized with the eight-species polyculture were also significantly faster than the rates achieved by the single most efficient species used in the study (95% confidence interval for polycultures, 1.45–4.01-fold faster than *Stigeoclonium*) (Fig. 1a, dashed line).

Species diversity did not alter the biomass-specific rates of nutrient uptake. There was no difference in the NO_3^- uptake rate per unit of

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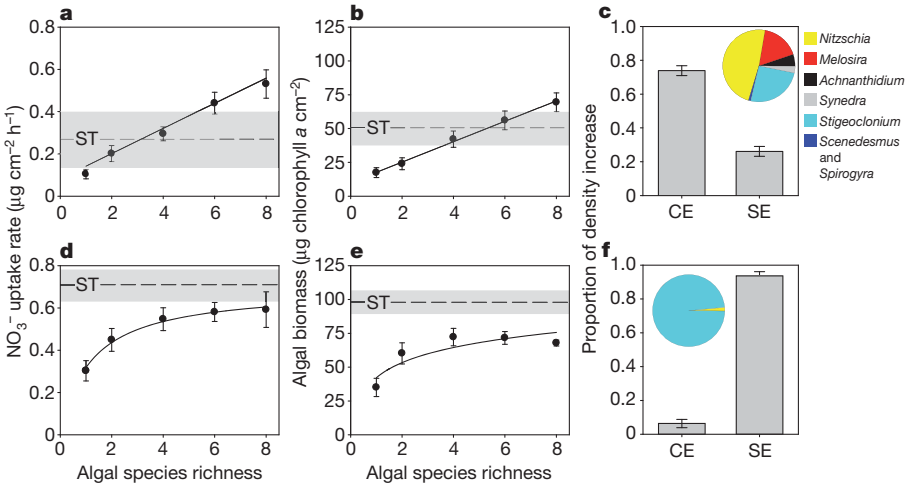

Figure 1 | Algal diversity effects on NO_3^-, algal biomass and final population sizes. **a**–**c**, Heterogeneous streams, with flow varying spatially and habitats varying in successional age. **d**–**f**, Homogeneous streams, in which niche opportunities had been removed. Data are presented as mean ± s.e.m. of 24 replicates for monocultures, 15 replicates for 2–6 species polycultures and 6 replicates for 8-species polycultures. Best fitting functions (Table 1) are plotted as solid lines. The horizontal line and the grey shaded area show mean ± s.e.m. for *Stigeoclonium*, which achieved the highest values of all of the monocultures. **c**, **f**, The proportion of increased polyculture cell densities driven by niche complementarity (CE) or selection effects (SE; that is, the influence of dominant species).

chlorophyll across the levels of richness ($P = 0.38$, linear mixed effects model, see Methods). However, a significant proportion of the variation in NO_3^- uptake could be explained by differences in the total algal biomass among streams (μg N cm^{-2} h^{-1} = 0.08 + (0.01 × μg chlorophyll *a* cm^{-2}), $P < 0.01$, $R^2 = 0.56$). Algal biomass was a linear function of diversity across the range of richness used in the study, increasing by 7.67 μg chlorophyll *a* cm^{-2} for each additional species (Table 1 and Fig. 1b). Increased algal biomass, and consequently the higher rate of NO_3^- uptake, led to a strong relationship between algal species richness and the total amount of nitrogen stored in the biofilm at the end of the experiment (μg [^{15}N + ^{14}N] cm^{-2} = 45 + (15 × richness), $P < 0.01$, $R^2 = 0.45$).

Two lines of evidence suggest that the effects of algal diversity on NO_3^- uptake and storage were driven by niche differences among species. First, if the frequency of disturbance and the spatial variation in flow represent axes of a species niche, then ecological theory predicts that different species should dominate different areas of this two-dimensional niche space[20,23]. Detailed examination of the algal population sizes showed that different morphological forms of algae dominated unique, complementary habitats in the streams (Fig. 2). High-velocity habitats were dominated by single-celled diatoms that grow prostrate to a surface in a way that is resistant to displacement by shear (for example, *Achnanthidium* and *Synedra*). By contrast, low-velocity habitats were dominated by large, filamentous algae that are susceptible to shear (for example, *Stigeoclonium* and *Melosira*). Fast-growing diatoms (such as *Achnanthidium* and *Nitzschia*) dominated habitats that had experienced recent disturbance. These early successional species were replaced by larger colonial, filamentous or slow-growing species in habitats that were less frequently disturbed (for example, *Spirogyra*, *Stigeoclonium* and *Synedra*). Differential habitat use by species led to a phenomenon called 'over-yielding' in algal polycultures, with five species achieving higher cell densities than would be expected on the basis of their initial proportional density (see Methods). As a result, more than 80% of increased cell densities in polycultures were driven by niche complementarity (Fig. 1c), which is enhanced when species use habitats or resources in ways that are either unique or synergistic[24].

The second line of evidence that suggests niche differences are responsible for increased NO_3^- uptake stems from the findings when niche opportunities were experimentally removed. Ecological theory predicts that when niche opportunities are eliminated from a system, the effects of biodiversity should disappear, and ecological processes should become dominated by a single, competitively superior species[20]. This is exactly what was observed. When the same algal species were grown in streams that had been forced to be spatially homogeneous (with near-bed flow velocity set to a uniform 22 cm s^{-1}, the median velocity of heterogeneous streams) and in which patches were never disturbed over the course of the experiment (all patches ~50 days old, with no variation in successional age), the previously observed effect of algal species richness on NO_3^- uptake rates disappeared. In these

Table 1 | Effects of algal diversity on rates of NO_3^- uptake and algal biomass

Dependent variable	Heterogeneous streams, with niche opportunities*				Homogeneous streams, with niche opportunities removed†			
	a	*b*	AIC	W_i	*a*	*b*	AIC	W_i
NO_3^- uptake rate (μg cm^{-2} h^{-1})								
Linear, $y = a + b \times S$	**0.05**	**0.06**	**−59.99**	**0.58**	0.34	0.05	8.73	0.00
Log, $y = a + b \times \log(S)$	0.09	0.19	−59.32	0.42	0.35	0.17	2.50	0.04
Hyperbolic, $y = a \times S/(b + S)$	0.06	−3.34	32.54	0.00	**0.77**	**1.17**	**−4.13**	**0.96**
Algal biomass (μg chlorophyll *a* cm^{-2})								
Linear, $y = a + b \times S$	**9.77**	**7.67**	**663.39**	**0.62**	42.79	6.11	716.44	0.02
Log, $y = a + b \times \log(S)$	14.30	22.58	664.41	0.37	**43.23**	**21.41**	**708.53**	**0.98**
Hyperbolic, $y = a \times S/(b + S)$	115.84	6.57	674.23	0.00	97.23	1.23	720.77	0.00

Measurements were taken in heterogeneous streams, where spatial variation in flow and periodic disturbances generated unique ecological niche opportunities, and homogenous streams, where niche opportunities had been intentionally removed by making all habitats physically uniform. Data were fit to linear, decelerating (log) and saturating (Michaelis–Menten hyperbolic) functions to characterize the general form of the relationship between algal richness and each response variable. The Akaike information criterion (AIC) was used to estimate Akaike weights, W_i, which give the relative likelihood of each model given the data. Highest relative likelihoods are in bold. *S*, species richness of algae.

* See also Fig. 1a–c.

† See also Fig. 1d–f.

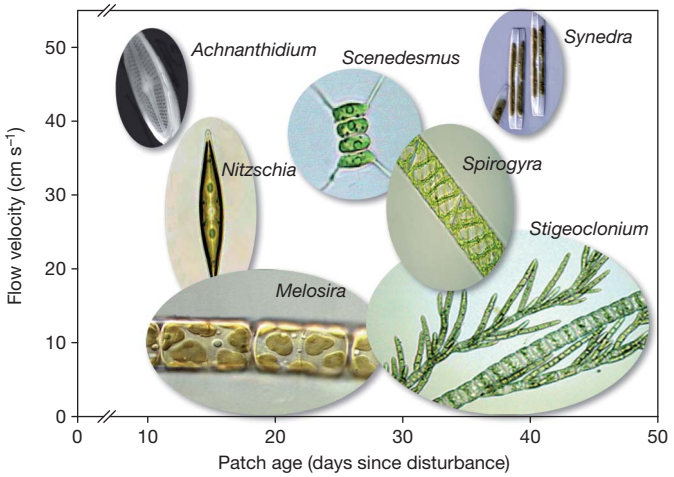

Figure 2 | Niche partitioning by algae. The ovals show the mean (centre of image) ± 95% confidence interval (boundary of image) of cell densities along two axes of a species niche (successional age of habitat and near-bed velocity). Filamentous algae that are susceptible to shear (*Melosira* and *Stigeoclonium*) were abundant in low-velocity habitats. Single-celled diatoms that grow prostrate to a surface (*Achnanthidium* and *Synedra*) achieved the highest densities in high-velocity habitats. Early successional habitats were dominated by small diatoms with fast rates of growth (*Achnanthidium* and *Nitzschia*), whereas late successional habitats were dominated by slow-growing cells, colonies or filaments (*Stigeoclonium*, *Spirogyra* and *Synedra*). *Navicula* is not plotted, because it failed to establish itself in polyculture despite growing in monoculture.

homogeneous streams, NO_3^- uptake rates and algal biomass were both positive but quickly decelerating functions of algal richness (Fig. 1d, e and Table 1). The most diverse polycultures took up NO_3^- at a rate that was twofold faster than the average monoculture, but these rates were significantly slower than that of the single most efficient species grown in monoculture (95% confidence interval for polycultures, -0.18 to $-0.21\times$ values for *Stigeoclonium*, $P = 0.04$, one-sided *t*-test). In these streams, more than 90% of the increased cell density in polycultures was driven by species-specific selection effects, which occur when initially diverse systems become dominated by a single, competitively superior taxon (in this case the filamentous green alga *Stigeoclonium*) (Fig. 1f). Such results suggest that in a homogeneous environment, the loss of niche opportunities led to reduced diversity and caused NO_3^- uptake to be controlled more by a single species than by algal species richness.

The results of this study build on previous research efforts by providing direct experimental evidence that ecological niche differences among species allow diverse communities to increase their uptake and tissue storage of nitrate. The specific linear responses documented in this study might not be expected to extrapolate to conditions in the field, where experiments performed with more species often show nonlinear responses[22]. Even so, this study is important because it confirms one of the fundamental mechanisms that has long been presumed to underlie the effects of diversity on ecological processes in ecosystems that range from the simple to the complex. That mechanism—niche partitioning—arose in this system because different species were best adapted for different habitats in a stream. These adaptations were expressed only when environmental conditions were dynamic in space and/or time and when heterogeneity provided ecological opportunities for species to coexist. Small, adnate forms of algae, which are known to be resistant to shear, dominated high-flow environments, whereas large, filamentous species that are prone to shear dominated low-flow environments. Fast-growing species dominated disturbed habitats, whereas slow-growing, competitively superior species dominated late successional habitats. Differences in ecological form allowed species to occupy unique and complementary habitat types in a stream. In turn, streams with diverse communities of microalgae had a greater capacity to remove NO_3^- than did streams with fewer numbers of species.

NO_3^- is one of the most abundant pollutants worldwide[13,14]; therefore, one implication of this study is that biodiversity could have a role in sequestering pollutants from natural environments. However, an important caveat to this conclusion is that nutrient pollution itself is known to reduce biodiversity, both through loss of species and through increased dominance of certain types of primary producer (for example, *Cladophora*). As a result, there is the potential for dual causality, whereby high nutrient loading of ecosystems reduces biodiversity, while the existing diversity reduces nutrient concentrations. How this feedback balances out to control diversity and nutrient levels in rivers that are increasingly being homogenized by human activities (for example, damming and altered flow regimes[29]) is an area of active research[30]. Nevertheless, the results of this study suggest two points. First, in those ecosystems where high nutrient loading reduces diversity, attempts to restore nutrient cycling to pre-disturbance conditions will probably be hampered by the irreversible loss of species. Second, in those ecosystems where it is possible to maintain species diversity despite nutrient loading, biodiversity may help to buffer ecosystems against the ecological impacts of nutrient pollution. Buffering against nutrient pollution will require not only the conservation of biodiversity, but also conservation of the forms of environmental heterogeneity that create niche opportunities and allow species to coexist.

METHODS SUMMARY

The species used for this study included eight forms of diatom and green alga that are among the most widespread and abundant species in North American streams (Supplementary Tables 1 and 2). The experiment was performed in the stream flume facility at the University of California Santa Barbara. This facility contains 120 recirculating laboratory 'flumes' (Supplementary Fig. 1), which are widely used for laboratory studies of lotic algae because they allow high replication rates, long-term population dynamics and large population sizes (Supplementary Information).

Algal diversity (one, two, four, six or eight species) was manipulated as a substitutive design (constant initial cell density of 10,000) in flumes that had two types of growth environment: heterogeneous and homogeneous. Heterogeneous flumes were constructed such that habitats within a stream had a wide range of near-bed velocities (from $<2\,cm\,s^{-1}$ to $55\,cm\,s^{-1}$), as well as disturbance regimes that allowed habitats to differ in successional age (from 5 to 50 days old). Homogeneous streams were constructed so that near-bed flow velocity was uniform ($22\,cm\,s^{-1}$, the median value for heterogeneous streams), and habitat patches were never disturbed. Thus, heterogeneous flumes had two forms of environmental variation that provided ample opportunity for species to express niche differences. By contrast, homogeneous flumes had no obvious spatial or temporal variation that might allow the expression of niche differences.

After allowing biofilms to reach a steady-state biomass, ^{15}N-enriched $NaNO_3$ (60% ^{15}N) was added to flumes in concentrations sufficient to increase $^{15}N/^{14}N$ ratios in the dissolved pool by 1.88-fold, but changing total NO_3^- by just 1.004-fold. After incubation, biofilms were destructively sampled to measure the amount of ^{15}N that had been sequestered by the biofilm and to estimate the final sizes of the algal populations.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions B.J.C. designed the study, collected data with the assistance of those mentioned in the Acknowledgements, analysed the data and wrote the paper.

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METHODS

Species pool. The algae used in the experiment included five species of Bacillariophyceae (the diatoms *Achnanthidium minutissimum*, *Melosira varians*, *Navicula cryptocephala*, *Nitzschia palea* and *Synedra ulna*) and three Chlorophyceaen green algae (*Scenedesmus quadricauda*, *Stigeoclonium* sp. and *Spirogyra* sp.). Although these taxa do not necessarily represent a random subset of all stream algae (a limited proportion of algal species exist in culture), they do rank among the most common and abundant types of primary producers in rivers throughout North America (Supplementary Table 1). In addition, representatives of these genera co-occur across a large proportion of rivers (Supplementary Table 2). As such, the study is representative of algal species that co-occur in many streams.

These species have a variety of morphologies, which are thought to influence the type of habitat the species occupy (Supplementary Fig. 1 and Supplementary Table 1). For example, *Achnanthidium* and *Synedra* are single-celled diatoms that attach prostrate to a growth surface, a function that is thought to be an adaptation to living in high shear environments[26,31,32]. By contrast, *Melosira*, *Stigeoclonium* and *Spirogyra* are large, filamentous algae that are known to be susceptible to drag and displacement by high shear stress[26,31]. Some of the species have fast rates of cell division and a high potential for colonization (for example, *Achnanthidium* and *Nitzschia*); as such, they often dominate early successional habitats after a disturbance[25,27,33,34]. Others are slow growing and tend to dominate late successional communities (for example, the diatom *Synedra*, the colonial alga *Scenedesmus* or the filamentous alga *Stigeoclonium*)[25,27,33].

Stream mesocosms. Algal diversity was manipulated in recirculating laboratory flumes ($0.5 \times 0.1 \times 0.1\ m^3$) in which discharge was controlled by 7-cm diameter propellers driven by a d.c. motor attached to a Hy3020E 3-A voltage controller (TekPower) (Supplementary Fig. 1). Biofilms in each flume were grown on a 200-cm^2 polyvinyl chloride (PVC) growth surface that had been roughened by sanding to facilitate colonization and growth. Growth surfaces were placed under a T5 Aquarium lighting fixture (Coralife) containing two 9-W, 10-K daylight-spectrum fluorescent lamps (Supplementary Fig. 1).

At the start of the experiment, each flume was sterilized with bleach and then filled with 13 l of 10% sterile Chu culture medium. Chu medium is widely used for growing freshwater algae and contains a suite of macronutrients and micronutrients at the stoichiometric ratios required by green algae and diatoms[35]. Each mesocosm was treated as a 'quasi-chemostat' in which 10% of the water volume was replaced with fresh medium each week to replenish nutrients and reduce waste.

Recirculating flumes are a widely used model system for laboratory studies of lotic algae and invertebrates[36]. In addition to the high level of replication that can be achieved, experiments can be run with large population sizes. For example, final population sizes on the growth substrates in this study averaged 1.2×10^9 cells for algae grown together in polyculture. For comparison, the entire Brazilian Amazon ($>4 \times 10^6\ km^2$) is estimated to have 2.7×10^{11} trees that are greater than 10-cm diameter breast height[37].

Experimental design. This experiment manipulated two variables in factorial combination: algal species richness (levels, one, two, four, six and eight species) $\times$ type of growth environment (levels, heterogeneous and homogeneous). Flumes assigned to the heterogeneous growth environment were constructed to have two forms of spatial and temporal variation that are widely thought to influence the diversity and coexistence of stream organisms (Supplementary Fig. 1). Spatial heterogeneity was added by angling the biofilm growth surface vertically in a flume, creating a flow constriction that generated spatial variation in near-bed velocities ranging from $<2\ cm\ s^{-1}$ at the bottom of the ramp to $55\ cm\ s^{-1}$ at the top of the ramp (velocities were measured by dissolution of gypsum pellets whose weight loss had been calibrated to free stream velocity in these flumes using a 16-MHz Micro Acoustic Doppler Velocimeter (SonTek)). The abundance, distribution and diversity of stream primary producers are known to be influenced by a stream's flow velocity[25,27,32,34,38], and species have evolved a variety of adaptations to deal with near-bed shear stress, as well as to sequester nutrients from boundary layers that control the delivery of materials to cells[26,27]. In comparison to the heterogeneous growth environments, growth substrates in the homogeneous flumes were laid flat on the bottom of the streams so that near-bed velocity could be set to a uniform $22\ cm\ s^{-1}$ (which is the median velocity of the heterogeneous environment).

To generate temporal variation in the heterogeneous flumes, growth substrates were divided into 18 equally sized patches, each of which had a probability of 0.25 of being 'disturbed' in each week of the experiment. When a patch was selected for disturbance, algae growing in that patch were removed using a soft-bristled bottle brush. This treatment was designed to generate a spatial mosaic of patches ranging in successional age from 5 to 50 days old, which is typical of streams that experience periodic disturbances owing to flow and sediment movement[28,39–42]. For flumes assigned to the homogeneous treatment, none of the 18 patches was disturbed, so all patches were uniformly 50 days old at the end of the experiment.

Treatments of algal diversity were applied as a randomized complete block in which biofilms differing in richness were established in 50 flumes (25 heterogeneous and 25 homogeneous) at a time, and the experiment was then repeated in three temporal blocks for a total of 150 mesocosms (75 per growth environment). In each block of the experiment, I grew each of the eight species alone as monocultures, five combinations of species at each intermediate level of richness (two, four and six species) with species selected randomly from all possible combinations, and two replicates of eight-species polycultures. Thus, for either type of environment (heterogeneous or homogeneous), the three experimental blocks included 3 replicate flumes for every species grown alone in monoculture, 15 replicate flumes at each level of two, four or six species, and 6 replicate full species polycultures.

At the start of each block, species were inoculated in the flumes according to a replacement series (that is, substitutive) design in which a total of 10,000 cells were added to the water irrespective of species richness. Flumes were inoculated on day 1, as well as day 7, of each block to ensure successful establishment. Each block lasted for 6 weeks. Pilot studies that were performed immediately before this study indicated that the 6-week time frame was sufficient to achieve ~10–20 doublings of algal population size (depending on the species) and for the full (eight-species) polycultures to reach a steady-state biomass.

Measurements. At the end of the experiment, I performed a ^{15}N tracer study to measure the rates of NO_3^- uptake by the biofilms. ^{15}N-enriched-$NaNO_3$ (60% ^{15}N) was added to sets of ten flumes at a time in intervals that were staggered by 20 min. The additions were sufficient to increase the $^{15}N/^{14}N$ ratio in the dissolved NO_3^- pool by a factor of 1.88 (1.65-, 2.00- and 1.99-fold for blocks 1–3). By contrast, additions resulted in a negligible change to the total concentration of NO_3^-, which increased by just 0.5% (1.004-, 1.006- and 1.006-fold for blocks 1–3). Thus, the $^{15}NO_3^-$ served as a tracer for nutrient uptake.

Biofilms were allowed to accumulate $^{15}NO_3^-$ for 2.4 ± 0.4 h. Then, the lights were turned off for sets of ten flumes at a time to prevent photosynthesis, and the biofilms in these flumes were destructively sampled over 20 min to obtain three measurements: final population size, final algal biomass and ^{15}N uptake.

To estimate final population sizes and to determine how these varied among habitat types in a flume, 0.49-cm^2 subsamples of the biofilm were taken from each of the 18 patches using a razor blade. The subsamples were preserved in 3% formalin and later used to identify species and to estimate cell densities using a haemocytometer viewed under a BX41 compound microscope (Olympus). The biofilm remaining on the 200-cm^2 growth surface (lacking the 8.8 cm^2 used for the 18 subsamples) was then removed with a razor blade and brought to a constant volume, and additional subsamples were taken for the other two measurements.

To estimate the final algal biomass in a flume, two subsamples of the biofilm were taken. The first was filtered onto a 0.45-μm GF-F filter (Whatman), which was sealed in a 10-ml Falcon tube containing 90% ethanol and placed in a freezer to lyse the cells and extract the photopigments. Concentrations of extracted chlorophyll *a*, which is a widely used estimate of algal biomass[14], were analysed spectrophotometrically using previously described methods[43], together with previously reported light extinction coefficients[44] for extracts in ethanol. A second subsample of the biofilm was dried for 48 h at 60 °C, after which total dry mass was determined. The Pearson correlation coefficient relating chlorophyll *a* and biofilm dry mass was 0.89, indicating that these two measures give nearly identical information. Chlorophyll *a* is reported on in this study, because it is specific to algae (that is, it does not include the mass of heterotrophs such as bacteria) and is perhaps the most widely used measure of algal biomass.

^{15}N uptake was estimated from a final subsample of the biofilm, which was dried for 48 h at 60 °C. The sample was then ground with a mortar and pestle, and preweighed samples were packed into combustible tin capsules. All mass spectrometry was performed at the University of California Santa Barbara's Marine Science Institute analytical laboratory. Samples were analysed using a DeltaPLUS isotope ratio mass spectrometer (Finnigan). ^{15}N values were expressed as $\delta^{15}N$, calculated as $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1{,}000$, where $R = {}^{15}N/{}^{14}N$ ratio and $R_{standard}$ is the $^{15}N/^{14}N$ ratio in atmospheric N_2 (0.0036765). Del values were converted to atom per cent, *AP*, as given in the appendix of ref. 45: $AP = 100(\delta + 1{,}000)/[(\delta + 1{,}000 + (1{,}000/R_{standard})]$. Nitrogen uptake rates were then calculated using equation 6 from ref. 46: $N_{uptake}\ (\mu g\ cm^{-2}\ h^{-1}) = [(AP_{is} - AP_{ns})/(AP_{ic} - AP_{ns})](N/t)$, where AP_{is} is the atom per cent of ^{15}N in the biofilm after incubation, AP_{ns} is the atom per cent of ^{15}N in algae before incubation (determined by analysis of three replicate samples from each algal batch culture used to inoculate the flumes), AP_{ic} is the atom per cent of ^{15}N in the dissolved phase at the beginning of the incubation (which was assumed to be in equilibrium

with air), N is the total nitrogen in the biofilm after incubation ($\mu g\ cm^{-2}$) and t is the incubation time in h.

Data analyses. N_{uptake} rates and algal biomass were both modelled as a function of algal species richness using three functions that have commonly been used to describe relationships between biological diversity and ecological processes in the literature[22]. These include a linear function, a positive but decelerating function (log) and a positive saturating function (the Michaelis–Menten hyperbolic equation). Parameter estimates were generated by maximum likelihood, using mixed model analyses that included blocking as a random effect. Akaike information criteria were used to judge the relative fits of the functions to the data[47].

It is methodologically impossible to distinguish how much ^{15}N or chlorophyll *a* is incorporated into the cells of different species grown together in a polyculture, as there is no practical way to separate the species physically. However, it is possible to distinguish between two factors that contributed to the final population densities of species in polyculture using an approach developed in ref. 24. With this formula, any difference in the biomass or density of two levels of species richness, ΔY, can be statistically partitioned into two additive components:

$$\Delta Y = N\overline{\Delta RY}\overline{M} + [Ncov(\Delta RY, M)] \quad (1)$$

where N is the number of species in a polyculture, RY is the relative yield (which can be based on biomass or density) of species in a polyculture and M is the yield of species in a monoculture. The notation ΔRY refers to the deviation in relative yield from some expected value, which is usually taken to be the proportional inoculation density of a species. For a replacement-series design such as that used here, $\Delta RY = I/N$, where I is the total inoculated density and N is the number of species in polyculture. In equation (1), the first term on the right-hand side measures what is commonly referred to as the complementarity effect (CE). Complementarity is positive whenever species yields in a mixture are, on average, higher than those that would be expected from the weighted average yield of the species in monoculture. This can occur when species use habitats or resources in ways that are complementary to one another, such as through niche partitioning, facilitation or other interactions that enhance species population sizes and/or per capita growth[48,49]. The second term on the right-hand side of equation (1) measures what is referred to as the selection effect (SE). Positive selection occurs if species with higher-than-average monoculture yields competitively dominate the polycultures.

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