Producer–decomposer co-dependency influences biodiversity effects

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Producers, such as plants and algae, acquire nutrients from inorganic sources that are supplied primarily by decomposers whereas decomposers, mostly fungi and bacteria, acquire carbon from organic sources that are supplied primarily by producers. This producer–decomposer co-dependency is important in governing ecosystem processes[1–4], which implies that the impacts of declining biodiversity on ecosystem functioning[5–7] should be strongly infuenced by this process. Here we show, by simultaneously manipulating producer (green algal) and decomposer (heterotrophic bacterial) diversity in freshwater microcosms, that algal biomass production varies considerably among microcosms (0.0–0.67 mg ml^{-1}), but that neither algal nor bacterial diversity by itself can explain this variation. Instead, production is a joint function of both algal and bacterial diversity. Furthermore, the range in algal production in microscosms in which bacterial diversity was manipulated was nearly double (1.82 times) that of microcosms in which bacterial diversity was not manipulated. Measures of organic carbon use by bacteria in these microcosms indicate that carbon usage is the mechanism responsible for these results. Because both producer[8] and microbial diversity[9] respond to disturbance and habitat modification, the main causes of biodiversity loss[8], these results suggest that ecosystem response to changing biodiversity is likely to be more complex than other studies[5–7] have shown.

Eukaryotic, photo-autotrophic producers generally cannot acquire carbon or nutrients from organic sources, but must transform inorganic carbon, such as CO_2, and inorganic nutrients, such as NO_3, into the organic materials needed for growth and reproduction (Fig. 1). Heterotrophic decomposers, in contrast, cannot acquire carbon from inorganic sources, but can transform organic carbon from sources supplied by producers, either as exudates[4,10–13] or as dead organic matter, and can acquire nutrients from organic or inorganic forms (Fig. 1). Decomposers transform organic nutrients into inorganic forms (mineralization), a main pathway of inorganic nutrients to producers[2,14] (Fig. 1). The asymmetry in metabolic capabilities results in a mutual co-dependency that inextricably links producers with decomposers and forms the foundation of material cycling in most solar-based ecosystems (Fig. 1). Although these links between autotrophic production, producer biomass, decomposer production and decomposer biomass are well studied (see for example refs 1, 4, 15, 16), little is known about the role of producer and decomposer diversity in these processes[17–19].

To test whether this producer–decomposer co-dependency influences producer diversity impacts on ecosystem functioning, we simultaneously manipulated unicellular green algal (producer) species richness (S_A) and heterotrophic bacterial (decomposer) species richness (S_B) in 116 freshwater, microbial microcosms. All microcosms contained bacteria, but our manipulations supplemented bacterial diversity by adding up to 12 additional species. All microcosms were inoculated weekly with the initial complement to ensure that treatment-designated species were present throughout the experiment. We measured algal production (standing algal biomass), decomposer production (standing microbial biomass) and carbon-source usage as response variables. Carbon source usage was assayed by monitoring the consumption by bacterial communities in each microcosm of 95 different organic carbon sources that span a wide breadth of bacterial metabolic capabilities[20]. Algae were removed by filtration before the assay, and assays were conducted in the dark to ensure that carbon use was strictly a factor of bacteria present in the microcosm at the time of measurement.

In microcosms where bacteria were not added, algal production showed a positive association with S_A ($R^2 = 0.49$, P , 0.05). This result agrees with studies that manipulate only producer diversity (for example, see refs 5, 20).

In contrast, where bacterial species were added, algal production was significantly affected by the interaction between S_A and S_B (S_A p S_B) (Table 1, Fig. 2). Bacterial production did not show a significant response to S_A, but S_B and S_A p S_B were significant (Table 1, Fig. 2). S_A p S_B affected total (algal plus bacterial) production (Table 1), although this is probably due to the disproportionate influence of algal biomass on total biomass (Fig. 2). Scaling algal and bacterial biomass to a maximum value of 1, and examining the ratio of these scaled abundances to avoid the disproportionate effect of algal biomass showed a significant S_A p S_B effect (Table 1).

The number of carbon sources used by bacteria was also affected by S_A and S_B, but not S_A p S_B (Table 1, Fig. 2). The number of carbon sources used showed a significant positive correlation with S_B ($R = 0.39$, P , 0.01), but no significant correlation with either S_A ($R =$ 2 0.18, $P = 0.15$) or total microcosm biomass ($R =$ 2 0.15, $P = 0.51$) was observed. These results indicate that the diversity of carbon sources used in a microcosm is a function of S_B. Although the diversity of carbon sources produced is expected to correlate with S_A, no correlation was expected between S_A and the number of carbon sources used because producers do not use organic carbon (Fig. 1). The significant treatment effects of S_A on the mean number of carbon sources used by bacteria (Table 1), however, indicate that the number of organic carbon sources used is indirectly affected by the impacts of producer diversity on bacterial communities.

The effects of S_A and S_B on numbers of organic carbon sources used by the bacterial communities indicate that the diversity impacts on production may be attributable to carbon-based producer–decomposer interactions that are well known in nature. A significant fraction (5–50%) of the organic carbon produced by

Table 1 Effects of variation in algal species richness, bacterial species richness and the interaction between these factors on microcosm properties

SOURCE	S_A			S_B			S_A p S_B		
	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Algal biomass	3	13.6	, 0.001*	4	4.5	, 0.01*	12	5.4	, 0.001*
Bacterial biomass	3	2.5	0.065	4	5.6	, 0.01*	12	3.2	, 0.01*
Total biomass	3	13.3	, 0.001*	4	3.9	, 0.01*	12	5.8	, 0.001*
Ratio of standardized bacterial to algal biomass	3	45.9	, 0.001*	4	10.0	, 0.001*	12	8.6	, 0.001*
No. of carbon sources used	3	7.2	, 0.01*	4	4.6	, 0.01*	12	1.7	0.118

The statistical test used was two-factor analysis of variance (ANOVA). The ratio of standardized bacterial to algal biomass was arcsine-square root transformed. Standardization involved scaling both maximum algal and maximum bacterial biomass to 1 to minimize the influence of algal biomass which was generally larger than bacterial biomass. S_A, algal species richness; S_B, bacterial species richness; S_A p S_B, interaction. $F = F$ value. P = significance. d.f. = degrees of freedom. Degrees of freedom for error term in all analyses was 59.
* Statistical significance.

plant and algae is exuded and consumed by decomposers in nature[2,4,12], and the remaining portion is eventually consumed as dead organic matter (although some fraction may become recalcitrant as humic substances or lost because of burial[21]). This organic carbon is a rich variety of complex organic compounds[3,4,11,15] that is matched by an equally diverse ability of decomposer microbes to consume these compounds[22]. Our findings are congruent with these observations from nature, thereby supporting bacterial exploitation of organic carbon sources as the link between decomposer diversity and algal production. Stoichiometric relationships suggest that such feedbacks are both carbon and nutrient based[3,23], thus the complexity of producer–decomposer feedbacks on diversity–ecosystem relationships may be even stronger than we observed if nutrients vary.

These results support a potentially strong influence of the producer–decomposer co-dependency on ecosystem response to changing biodiversity. Only in the absence of experimental manipulation of decomposer diversity was producer production positively associated with producer diversity; otherwise production was a joint function of producer and decomposer diversity. Producer-only studies (for example, see refs 5, 7, 18) are likely to underestimate the magnitude and complexity of biodiversity impacts. Simultaneous variation in decomposer diversity is likely to accompany most changes in diversity that are caused by disturbance or habitat modification[8,24]. Such linkages as the producer–decomposer co-dependency among species implies that the preservation of biodiversity as a means for preserving, restoring and managing ecosystem functioning requires a multitrophic approach in which decomposers, producers and other trophic groups[25,26] are integrated in both research and practice. M

Methods

Microcosms

Microcosms consisted of disposable, sterile 100-mm diameter × 25-mm deep polystyrene Petri dishes each filled with 50 ml of growth medium (Carolina Biological Supply) (for additional detail, see ref. 6). Chamber conditions were 20 °C with a 14:10 h, light:dark photoperiod.

Experimental design

Algal species richness treatments were 0, 1, 2, 4 and 8 species chosen randomly from *Chlamydomonas reinhardti*, *Carteria olivieri*, *Closterium* sp., *Eromosphaerum* sp., *Euglena gracilis*, *Netrium* sp., *Phacus* sp. and *Trachelomonas* sp. Bacterial species richness treatments were 0, 1, 2, 4, 8 and 12 species chosen from a pool consisting of *Aeromonas sobria*, *Aquaspirillum iterosonii*, *Aq. serpens*, *Aq. sinuosum*, *Bacillus cereus*, *B. megaterium*, *B.*

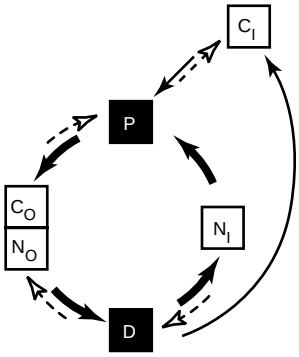

Figure 1 The fundamental producer–decomposer co-dependency common to most ecosystems. Filled boxes represent biomass and open boxes represent material pools. The biomass compartments shown are producers (P) and decomposers (D). The material pools are organic carbon (C_0), inorganic carbon (C_1), organic nutrients (N_0) and inorganic nutrients (N_1). Solid arrows indicate major flows, whereas dashed, open arrows indicate minor flows such as producer resorption of carbon (known to occur in some plants) and producer respiration. Producers are dependent on decomposers for uptake of inorganic nutrients, whereas decomposers are dependent on producers for organic materials. The cycling of material (counterclockwise array of solid arrows in centre) results from these fundamental activities. (For simplicity, we have treated this system as a system closed to internal and external inputs and outputs.)

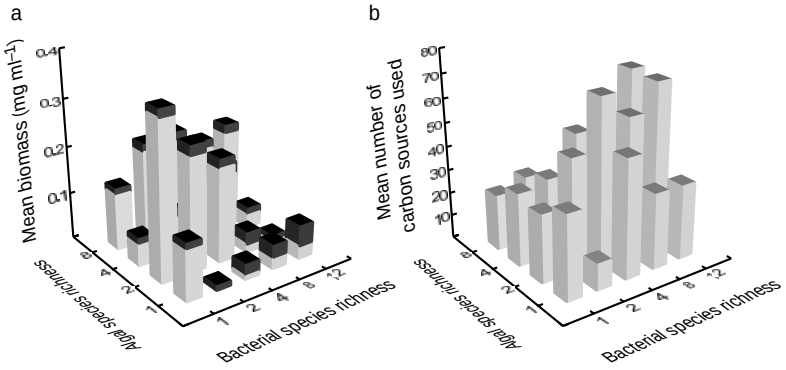

Figure 2 Relationships between producer diversity, bacterial diversity, standing biomass and carbon-source usage. **a**, Mean biomass in microcosm replicates containing different numbers of algal and bacterial species after 4 weeks. Bars are divided with top, darker portion showing the fraction of biomass found in bacterial populations. The highest proportions of bacterial biomass occur when bacterial species richness is high and algal species richness is low, whereas higher proportions of algal biomass occur when algal species richness is intermediate to high and bacterial species richness is low. **b**, Mean number of carbon sources used in replicate microcosms for each diversity treatment. Carbon sources consisted of 95 unique organic substrates used by bacterial assemblages taken from microcosms. All species of bacteria were freshwater species adapted to culture conditions, thus the problem with unculturables does not apply here. The diversity of carbon sources used is higher when bacterial species richness is high and algal species richness is high to intermediate.

subtilis, *Enterobacter cloacae*, *Ochrobactrum anthropi*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *S. liquefaciens*. We did not construct 0 algae/0 bacteria microcosms. This design yielded 118 microcosms ($S_A = 5$ levels 3 $S_B = 6$ levels 3 4 relicates 2 $4S_A$ or $S_B = 0$). Algal and bacterial stock cultures were maintained in the same medium used in microcosms. We carried out four replicates per species composition, and replicates were dispersed among four separate growth chambers to avoid pseudoreplication. One hundred algal cells in total were added to each microcosm (for example, 100 cells per species in monocultures, 50 cells per species in two species treatments). One millilitre of bacteria culture(s) was added to each microcosm (for example, 1 ml for each species in monocultures, 0.5 ml of each species in two species treatments). Note that the initial biomass in the small inoculum was insignificant, thus final measures of biomass were used to estimate production.

Measurements

Algal biomass was determined from algal biovolume[27], whereas bacterial biomass was determined from conversion of densities determined by DAPI enumeration[28] in 5-ml samples. Measurements were made after 4 weeks (algal densities stabilize after two weeks[6,29]). Sole source carbon usage was determined by Biolog plate methods[19]. Samples were filtered with 20-mm polycarbonate membrane filters to remove algae before plating and plates were incubated at 20 °C for 24 hours. A M3E Biolog plate reader was used to read microplates. The bacterial species that we selected are well adapted to laboratory conditions and pre-tests showed that they grew well in microplates; thus problems associated with unculturable species from environmental samples[30] do not apply to our application.

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The mouse *Dreher* gene *Lmx1a* controls formation of the roof plate in the vertebrate CNS

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In the vertebrate central nervous system (CNS), a cascade of signals that originates in the ectoderm adjacent to the neural tube is propagated by the roof plate to dorsalize the neural tube[1]. Here we report that the phenotype of the spontaneous neurological mutant mouse *dreher* (*dr*)[2–5] results from a failure of the roof plate to develop. Dorsalization of the neural tube is consequently affected: dorsal interneurons in the spinal cord and granule neurons in the cerebellar cortex are lost, and the dorsal vertebral neural arches fail to form. Positional cloning of *dreher* indicates that the LIM homeodomain protein, Lmx1a, is affected in three different alleles of *dreher*. *Lmx1a* is expressed in the roof plate along the neuraxis during development of the CNS. Thus, *Lmx1a* is required for development of the roof plate and, in turn, for specification of dorsal cell fates in the CNS and developing vertebrae.

Spontaneously generated mutant mice provide a powerful tool for analysing genetic and epigenetic mechanisms of CNS development[6]. To define further the postnatal phenotype of dr^J/dr^J mice, we analysed the cytoarchitecture of the cerebellum and spinal cord. In the cerebellum the vermis was absent in dr^J/dr^J mice (Fig. 1a, b) and fewer lobes were present at the midline of dr^J/dr^J as compared with wild-type mice (Fig. 1c, d). Although lobe patterning was disrupted, the three layers of the cerebellum were present at the cellular level (Fig. 1d). In ventral areas of the cerebellar system, only the pontine nucleus was reduced in size in dr^J/dr^J mice (Fig. 1e, f). In more caudal regions of the CNS, the spinal cord had a flattened appearance and cell density appeared to decrease in the dorsal spinal cord (Fig. 1g, h). Examination of the skeleton of the caudal region of the neural tube revealed that the neural arches failed to fuse on the dorsal midline and bone density appeared to be reduced (Fig. 1i, j).

An intercross between dr^J and wild-type MOLF/Ei mice established a detailed genetic and physical map surrounding the *dreher* locus. From 738 informative meioses, four recombination events defined the dr^J locus to a 0.68-cM interval. The closest proximal and distal markers were *D1Mit109* (0.13 cM) and *D1Mit370* (0.54 cM), respectively. The genetic map of this region is shown in Fig. 2a, b. Although *D1Mit110* and the gene *RXR*g co-segregated with the dr^J

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