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LETTER

Food webs obscure the strength of plant diversity effects on primary productivity

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Abstract

Plant diversity experiments generally find that increased diversity causes increased productivity; however, primary productivity is typically measured in the presence of a diverse food web, including pathogens, mutualists and herbivores. If food web impacts on productivity vary with plant diversity, as predicted by both theoretical and empirical studies, estimates of the effect of plant diversity on productivity may be biased. We experimentally removed arthropods, foliar fungi and soil fungi from the longest-running plant diversity experiment. We found that fungi and arthropods removed a constant, large proportion of biomass leading to a greater reduction of total biomass in high diversity plots. As a result, the effect of diversity on measured plant productivity was much higher in the absence of fungi and arthropods. Thus, diversity increases productivity more than reported in previous studies that did not control for the effects of heterotrophic consumption.

Keywords

Biodiversity, dilution effect, pathogens, community ecology, ecosystem ecology.

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INTRODUCTION

One of the most important and controversial developments in ecology in recent decades has been the experimental demonstration of strong, direct effects of diversity on a wide array of ecosystem functions including biomass production, resistance to species invasions, ecosystem stability, disease suppression, pollinator abundances, and heterotroph abundance and richness (Hector *et al*. 1999; Tilman *et al*. 2001; Hector & Bagchi 2007; Naeem *et al*. 2010, 2012; Scherber *et al*. 2010; Isbell *et al*. 2011). In part, the increased interest in quantifying the direct effects of biodiversity on critical ecosystem processes has arisen from the rapid increase in the magnitude of human activities, such as ecosystem eutrophication and habitat fragmentation, that simplify ecosystems by decreasing local and regional biodiversity (Clark & Tilman 2008; Naeem *et al*. 2012; Borer *et al*. 2014).

The greater productivity associated with higher plant diversity lies at the core of the links between diversity and many other ecosystem functions (Hector & Bagchi 2007; Scherber *et al*. 2010; Isbell *et al*. 2011). However, studies investigating the effects of diversity on productivity, whether in experimentally assembled or naturally occurring plant communities, are typically conducted in the presence of heterotrophs, including a wide array of herbivores, predators, detritivores, pathogens and plant mutualists (e.g. pollinators or mycorrhizae). For example, most diversity experiments do not account for autotroph biomass removed by herbivores, pathogens, and other heterotrophs prior to sampling when quantifying primary productivity. Productivity measures that do not account for the effects of heterotrophs on productivity might bias the estimated magnitude of the effects of biodiversity on productivity (Thebault & Loreau 2003; Duffy *et al*. 2007; Maron *et al*. 2011; Schnitzer *et al*. 2011), because the abundance and diversity of many heterotrophic organisms vary along experimental plant diversity gradients (Mitchell *et al*. 2002; Scherber *et al*. 2010; Borer *et al*. 2012; Hertzog *et al*. 2016). For example, the abundance of foliar fungal pathogens has been shown to decline with plant diversity (i.e. the dilution effect; Mitchell *et al*. 2002; Keesing *et al*. 2006; Scherber *et al*. 2010). In contrast, arthropod and soil microbial abundance and diversity have been found to increase with plant diversity (Haddad *et al*. 2001; Bruno *et al*. 2008; Haddad *et al*. 2009; Scherber *et al*. 2010; Borer *et al*. 2012; Hertzog *et al*. 2016) (but see Koricheva *et al*. 2000), potentially creating an amplification effect in which heterotroph abundance and diversity is greatest in high diversity host communities (Keesing *et al*. 2006).

A directional change in abundance and diversity of heterotrophs along experimental diversity gradients could lead to a biased over- or under-estimation of the role of plant diversity in primary productivity, if the changes in heterotroph abundance or diversity translate into impacts on plant biomass (Thebault & Loreau 2003; Duffy *et al*. 2007; Maron *et al*. 2011; Schnitzer *et al*. 2011). However, there have been few attempts to assess whether these changes in abundance alter basic ecosystem functions such as productivity (but see Bruno *et al*. 2008; Maron *et al*. 2011; Schnitzer *et al*. 2011).

Despite the potential for shifting effects of heterotrophs along diversity gradients, the mechanisms proposed to explain the diversity effect on productivity rarely incorporate the

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effects of heterotrophic food webs (Berendse 1982; McKane *et al.* 1990; Tilman *et al.* 1996; Fargione & Tilman 2005; von Felten *et al.* 2009; Fornara & Tilman 2009; Reich *et al.* 2012; Mueller *et al.* 2013) (but see Thebault & Loreau 2003, 2006; Bruno *et al.* 2008; Maron *et al.* 2011; Schnitzer *et al.* 2011). Mechanisms by which increased plant diversity can lead to increased primary productivity fall into two broad categories: the complementarity effect and the selection effect (see also the related sampling effect; Loreau & Hector 2001; Fargione *et al.* 2007), each of which may be altered by the presence of heterotrophs. The selection effect emerges if plant species that are highly productive in monoculture are a major determinant of productivity in diverse communities assembled by random draws from a common species pool, as is the case for most biodiversity experiments (Loreau & Hector 2001; Fargione *et al.* 2007). These highly productive species have morphological and physiological traits that support their high biomass accumulation (Roscher *et al.* 2012), and these traits also could include defence against herbivores or effective acquisition of beneficial microbes (see review in Lind *et al.* 2013). Complementarity effects arise when niche differences among species moderate intra-specific competition and thus promote increased overall productivity in mixtures, or when facilitation increases resource availability or species abundances (Loreau & Hector 2001; Fargione *et al.* 2007). Well-documented processes leading to complementarity effects include among-species differences in growth-limiting nutrients, altered root distributions and positive feedbacks between productivity and soil nutrients (Berendse 1982; McKane *et al.* 1990; Tilman *et al.* 1996; Fargione & Tilman 2005; von Felten *et al.* 2009; Fornara & Tilman 2009; Reich *et al.* 2012; Mueller *et al.* 2013); however, differences in plant defence, pathogen transmission or plant-associated microbial communities that vary with total plot diversity could significantly contribute to complementarity effects and associated overyielding (van Der Heijden *et al.* 1998; Schnitzer *et al.* 2011; Kulmatiski *et al.* 2012).

If the net effect of heterotrophs on plant biomass changes with plant diversity, including heterotrophs in our understanding of diversity–productivity relationships could fundamentally alter our assessment of the magnitude and mechanisms that underlie the influence of plant diversity on primary productivity (Fig. 1) (Maron *et al.* 2011; Schnitzer *et al.* 2011). Our interpretation of these effects also depends on whether we are considering the proportional impacts (Fig. 1a) or absolute impacts of heterotrophs on plant biomass (Fig. 1b).

For example, reduced heterotroph impact with increasing diversity, consistent with patterns frequently found in fungal pathogens (Mitchell *et al.* 2002; Keesing *et al.* 2006; Scherber *et al.* 2010), would lead to an overestimation of the direct effects of plant diversity on productivity (difference between Lines 1 & 2 in Fig. 1) (Bruno *et al.* 2008; Maron *et al.* 2011; Schnitzer *et al.* 2011). In this case, removing consumers would increase the intercept and reduce the slope of the linear log-diversity log-productivity relationship (Fig. 1; Maron *et al.* 2011). In contrast, increased heterotroph impacts at high plant diversity, consistent with abundance patterns found in many arthropod and herbivore communities (Haddad *et al.* 2001, 2009; Bruno *et al.* 2008; Scherber *et al.* 2010; Borer *et al.* 2012), would lead to an underestimation of biodiversity effects

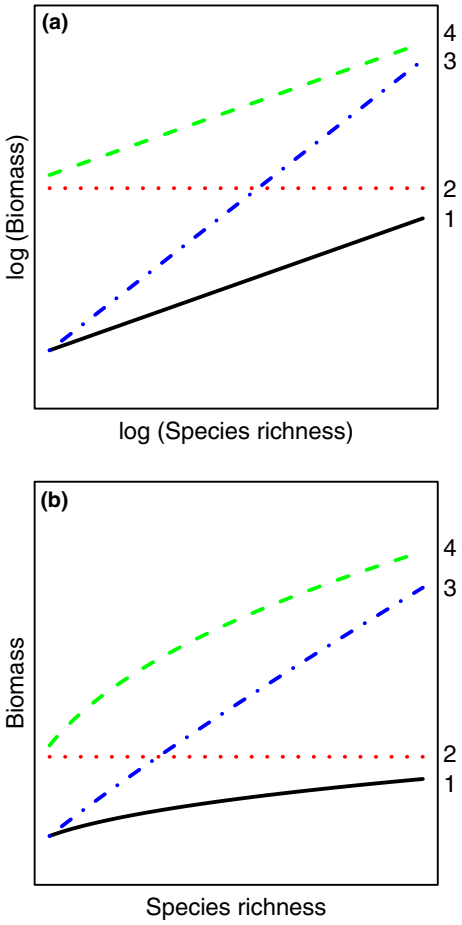

Figure 1 Alternative potential effects of diversity and consumers on plant productivity on both a log-log (a) and linear scale (b). The standard diversity productivity curve includes both the effect of plant richness and the heterotrophic food web on productivity (Line 1). If consumers reduce plant biomass by a constant proportion at all levels of plant richness (Line 4), the slopes of the diversity–productivity relationship will be similar with and without consumers in a log-log plot (Lines 1 and 4 in Panel a), but absolute biomass reduction by consumers will increase with species richness (the difference between Lines 1 and 4 in Panel b). If consumer effects decline with species richness (Line 2), the slope in the log-log plots will be lower in the absence of consumers (Line 2 in Panel a) than in their presence (Line 1 in Panel a). Conversely, if removal rates increase with species richness (Line 3), the slope in the log-log plots will be higher in the absence of consumer's treatment (Line 3 in Panel a) than in their presence (Line 1 in Panel a)). Finally, if consumption rates are negligible, there should be no difference between the control and removal plots (corresponding to Line 1).

on productivity (difference between Lines 1 & 3 in Fig. 1). In this case, removing heterotrophs would increase the slope of the linear log-diversity log-productivity relationship (Fig. 1; Maron *et al.* 2011).

It is also possible that heterotrophs have a constant proportional effect on plant biomass (Lines 1 & 4 in Fig. 1a), in which case the absolute impacts of heterotrophs on decreasing plant biomass would be higher in diverse communities (difference between Lines 1 and 4 in Fig. 1b). In the case of constant proportional effects, we would expect that removing heterotrophs would change the intercept but not the slope of

the linear log-diversity vs. log-productivity relationship (Fig. 1; Maron *et al*. 2011).

Heterotrophs also may have little effect on plant biomass, if they are generally well defended against a broad suite of enemies (Durrant & Dong 2004). Heterotroph removal could also be small if primary consumers, such as herbivores, are kept at low levels by increased predator abundance (Duffy *et al*. 2007; Haddad *et al*. 2009), or if the beneficial effects of mutualists, such as mycorrhizal fungi, root-associated beneficial bacteria, or pollinators, are counterbalanced by the negative effects of pathogens and herbivores (Kulmatiski *et al*. 2012).

While we have some understanding of the patterns of abundance of common heterotroph groups along plant diversity gradients, we currently have little understanding of the overall direction and magnitude of the food web impacts on plant biomass along experimental diversity gradients. For example, it is possible that removal of heterotrophs may actually decrease plant biomass if the effects of mutualists outweigh the effects of enemies (Morris *et al*. 2007). Further, it remains unclear whether the effects of the heterotrophic food web bias current estimates of biodiversity effects on primary productivity (Maron *et al*. 2011). Resolving this potentially large source of bias is important, because the diversity-productivity relationship underlies our understanding of a wide array of ecological processes and functions with implications for basic and applied science (Naeem *et al*. 2010, 2012). Our current knowledge does not inform whether it is necessary to include consumers to generate a more mechanistic and predictive understanding of the processes generating diversity–productivity relationships.

Here, we test whether removing important suites of heterotrophs alters biodiversity effects on productivity in a long-term plant diversity experiment (Tilman *et al*. 1996; Naeem *et al*. 2010, 2012). We measured below and aboveground biomass in plots from which we removed three broad groups of heterotrophs, soil fungi, foliar fungi, and arthropods, separately and in combination, from experimental plant communities established with 1, 4 and 16 grassland species. The plant diversity experiment was started in 1994 and the food web manipulations were begun in 2008. Using response data from 2010 to 2013, we address the following questions: (1) Is the direction and magnitude of the effect of each of these components of the heterotroph food web (individually and in combination) on plant biomass consistent with effects caused by consumer dilution or amplification? Specifically, do these heterotroph groups change the slope or intercept of the log-diversity log-productivity relationship?, and (2) Do these heterotroph groups change the strength of selection, complementarity, or the difference between the complementarity and selection effects (i.e. the *net biodiversity* effect) (Loreau & Hector 2001)?

MATERIALS AND METHODS

This experiment was conducted within a plant-diversity experiment established at the Cedar Creek Ecosystem Science Reserve (Tilman *et al*. 2001, 2006; Borer *et al*. 2015). The 168 – 9 m × 9 m plots were randomly assigned to species richness treatments ranging from 1 to 16 species, with the composition of each plot being a random subset of a pool of 18 native perennial species (four C4 grasses, four C3 grasses, four legumes, four forbs, and two woody species). By way of comparison, the 16 species plots in this experiment are representative of diversity levels found in high-quality, grassland ecosystems in the region in which this experiment was conducted, while the 1 and 4 species plots represent the range of more degraded grasslands or native biomass crops in this area (Tilman *et al*. 2012). Plots were seeded in May of 1994 and 1995 with a total of 15 g of seed per m^2, with the seed evenly divided among each species. The species richness treatments were maintained by weeding (Tilman *et al*. 2001, 2006).

In 2008, we established a food web manipulation experiment within 33 of the 9 × 9 m diversity plots with plant richness treatments of 1 (n = 15), 4 (n = 9), or 16 (n = 9) species. We randomly assigned each of five food web manipulations to five different 1.5 × 2 m subplots within the 9 × 9 m diversity plots. Treatments included: Control, Insecticide, Foliar Fungicide, Soil Fungicide or All Treatments in Combination (Insecticide, Foliar Fungicide and Soil Fungicide). The overall food web manipulation experiment was composed of 165 subplots (33−9 × 9 m plots each with five 1.5 × 2 m subplots) distributed across 15 treatment combinations (three plant diversity treatments crossed with five food web treatments).

All of the pesticides used for the food web manipulations were applied throughout the growing season, from mid April to the end of August each year. The foliar fungicide treatment was composed of Quilt (Syngenta Crop Protection, Inc., Greensboro, NC, USA), a combination of Azoxystrobin (7.5%) and Propiconazole (12.5%), applied every two weeks. The soil fungicide treatment was composed of monthly applications of Ridomil Gold SL (Syngenta Crop Protection, Inc., Greensboro, NC, USA), a soil drench fungicide containing Mefenoxam (45.3%). The insecticide treatment was composed of Marathon II (OHP, Inc., Mainland, PA, USA; 21.4% Imidacloprid) applied ever two weeks. One to two times each growing season, Malathion was applied instead of Marathon II to reduce the possibility for insecticide adaptation by the local insect populations. The treatments had strong effect on the incidence of herbivory and foliar pathogens, plant tissue chemistry, and composition of the plant communities (Borer *et al*. 2015).

It is possible that the pesticides caused direct effects on plant growth even in the absence of the target fungi and arthropods. Therefore, we performed a greenhouse experiment to test for the effects of the insecticide, foliar fungicide and soil fungicide on plant growth. A soil mix composed of 80% soil sourced from the field site (Cedar Creek Ecosystem Science Reserve) and 20% vermiculite was used for potting. Soil was coarsely sieved and the soil mix was homogenized and autoclaved. The experiment was conducted using two C4 grasses (*Andropogon gerardii* and *Schizichyrium scoparium*) and two legumes (*Lespedeza capitata* and *Lupinus perennis*). These four species are common at Cedar Creek and represent a wide range of the functional trait variability present in the pool of species in the diversity experiment (Craine *et al*. 2002; Borer *et al*. 2015).

A total of 60 pots were seeded with one of the four species (total 240 pots) with seeds obtained from Prairie Restoration Inc. Ten pots of each species were randomly assigned to each of six treatments (Foliar Fungicide, Insecticide, Soil Drench

fungicide, All Treatments, Foliar-Water Control and Soil-Water Control). All pots were watered every other day and treatments were applied once every 2 weeks. Leaf-water and soil-water controls were treated with water at the time of pesticide application. Pesticide application rates were calculated based on the surface area of the pots to match the field experiment, and the pesticides used in the experiment were the same as those used in the field experiment. Plants were allowed to grow for a total of 15 weeks. At the end of this period, the above and belowground parts of each individual were harvested, cleaned, oven-dried (at 70 °C for 48 h) and weighed to the nearest 0.01 g. We analysed the log-transformed root and shoot biomass using ANOVA.

From 2010 to 2013, we collected above- and belowground biomass annually at the time of peak biomass (early August) in each subplot of the experiment. Aboveground biomass was collected from a 1 × 0.1 m strip in each plot. A handheld clipper was used to cut all plants rooted in the strip as close to the ground as possible. The aboveground biomass was sorted to species, dried to a constant mass and weighed to the nearest 0.001 g. Belowground biomass was collected annually from three cores per plot (5 cm diameter and 30 cm depth). Soil was washed away from roots with water; roots were dried to a constant mass, and weighed to the nearest 0.001 g.

Complementarity, Selection and Net Biodiversity effects were calculated as in Loreau & Hector (Loreau & Hector 2001) and Fargione *et al.* (2007). In brief, the net biodiversity effect (NE) can be partitioned into a selection effect (SE) and a complementarity effect (CE). It is additive because NE = SE + CE. The net biodiversity effect is measured by the difference between the observed yield of a mixture and its expected yield based on monocultures. The selection effect is measured by the covariance between the monoculture yields of the species and the deviation from their expected relative yields in mixture. The complementarity effect is then calculated as CE = NE–SE. Our samples permitted calculation of complementarity and selection effects using only aboveground biomass, as this calculation requires biomass to be identified and sorted to species, which was not feasible for root biomass.

All models were fit using the lme function in R (v. 3.0.1; R Foundation for Statistical Computing, Vienna, Austria). The 9 × 9 m diversity plot, 1.5 × 2 m food web manipulations subplot, and sampling year were treated as random effects. Biomass and species richness were log transformed to account for the nonlinear saturating effects of diversity on biomass observed in many experiments (Fig. 1) (Scherber *et al.* 2010; Reich *et al.* 2012).

RESULTS

On average across all plots and diversity treatments, removing heterotrophs increased total plant biomass (aboveground plus belowground biomass) by 44%, from 957 ± 61 to 1378 ± 93 g m^{-2}; increased aboveground biomass by 32%, from 203 ± 14 to 268 ± 20 g m^{-2}; and increased belowground biomass by 47%, from 754 ± 51 to 1110 ± 80 g m^{-2} (mean ± 1 SEM) (Fig. 2; Table S1). Although the heterotrophic food web is composed of organisms that have positive (e.g. pollinators and mycorrhizae) and negative (e.g. herbivores and pathogens) effects on biomass, the observed reduction in plant biomass demonstrates that the net effect of these plant–heterotroph interactions was antagonistic.

Our greenhouse experiment demonstrated that these effects on plant biomass did not arise as side-effects of the pesticide applications on plant growth. When grown in sterile soil and in the absence of arthropods, there were no detectable effects of any of pesticide applications ($P > 0.25$) on root or shoot biomass treatments, when compared to their specific controls (Foliar Fungicide and Insecticide vs. Foliar-Water Control; Soil Drench fungicide vs. Soil-Water Control) or when all treatments were compared to an average of the two Controls.

We found that the total reduction in plant biomass caused by heterotrophs was much higher in the most diverse plant communities (Fig. 2b, d and f) reflecting an approximately constant proportional biomass reduction across the plant diversity gradient (Fig. 2a, c and e; Table S1). The combined removal of insects and both fungal groups increased total biomass by 220 g m^{-2} in the single species plots and 637 g m^{-2} in the 16 species plots. As a result, the increase in total biomass between the average 16 species and the single species plots was 35% higher in the plots receiving foliar fungicide, soil fungicide, and insecticide (an increase of 1614 g m^{-2}) than in Control plots (an increase of 1197 g m^{-2}).

Complementarity became increasingly important in high diversity plots, resulting in an increase in the net biodiversity effect (Fig. 3; Table S2) (Loreau & Hector 2001); however, heterotrophs did not alter the relative strength of selection and complementarity effects.

DISCUSSION

We found that consumers removed a constant proportion of total plant biomass at all levels of diversity which resulted in three times more plant mass being lost in high-diversity plots compared to monocultures (637 vs. 220 g m^{-2}). As a result, the direct effect of biodiversity on primary productivity was substantially higher than had been estimated by previous studies. In the absence of heterotrophs, total biomass increased 1614 g m^{-2} from 1 to 16 species, while in the presence of heterotrophs, it increased 1197 g m^{-2} from 1 to 16 species. Thus, measuring living plant biomass in the presence of arthropods and fungi underestimated the actual effect of plant species richness on plant biomass production (Fig. 1), because a large portion of the primary production had already been removed by consumers prior to measurement of the plants. This result is apparently quite general, as it is consistent with effects of marine grazers (Bruno *et al.* 2008). Taken together with previous work, these results suggest that greater diversity of primary producers leads to increased net primary production that simultaneously supports greater consumption, secondary production, and a more abundant and diverse heterotroph web (Bruno *et al.* 2008; Haddad *et al.* 2009; Borer *et al.* 2012).

The strongest effects of treatments on plant production arose from the foliar fungicide and insecticide treatments, and they acted on both above and belowground biomass. The reduction of root mass by aboveground herbivores and pathogens, points to the strong coupling of belowground and aboveground processes in grassland systems. Although soil

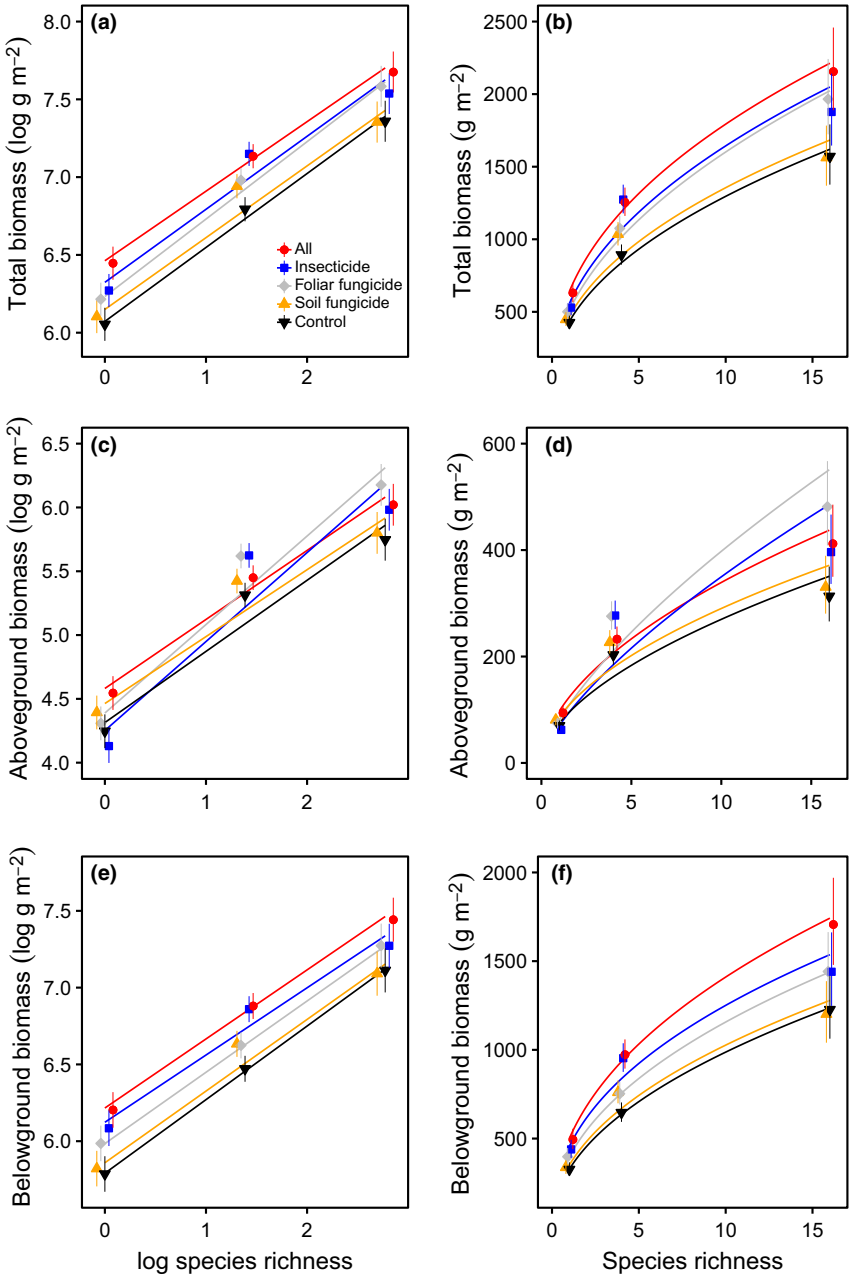

Figure 2 Effects of heterotroph removal on the total biomass, aboveground biomass and belowground biomass along an experimental diversity gradient. Heterotroph removal treatments were Control (black triangles), Foliar Fungicide (grey diamonds), Soil Fungicide (gold triangles), Insecticide (blue squares), or All Treatments in Combination (All; red circles). Error bars 1 standard error around treatment means. Responses are plotted showing (a, c and e) proportional effects (log biomass vs. log richness) and (b, d and f) absolute effects (biomass vs. richness).

fungicide had little effect on biomass, it had strong effects on the composition of the plant community leading to an overall increase in the abundance of forbs and legumes and reduced C3 grasses in our experiment (Borer *et al.* 2015).

Previous work has found that arthropod abundance tends to increase with increasing plant species richness (Haddad *et al.* 2001, 2009; Bruno *et al.* 2008; Scherber *et al.* 2010; Borer *et al.* 2012), and our work demonstrates that the higher abundance of arthropods is associated with a larger reduction in plant biomass in more diverse plant communities. It is not a foregone conclusion that increased arthropod abundance would translate directly to increased impacts on plant biomass, because many arthropods have direct or indirect positive impacts on plants (e.g. pollinators, predators and parasitoids), and the relative abundances of these functional guilds can vary with plant diversity (Koricheva *et al.* 2000; Haddad *et al.* 2009; Scherber *et al.* 2010). In the experiment in which this work was conducted, the total mass of herbivores increases with plant species richness (Borer *et al.* 2012), although the relative abundance and diversity of predators and parasitoids increases on a per individual basis (Haddad *et al.* 2009). The results presented here suggest that the effect of

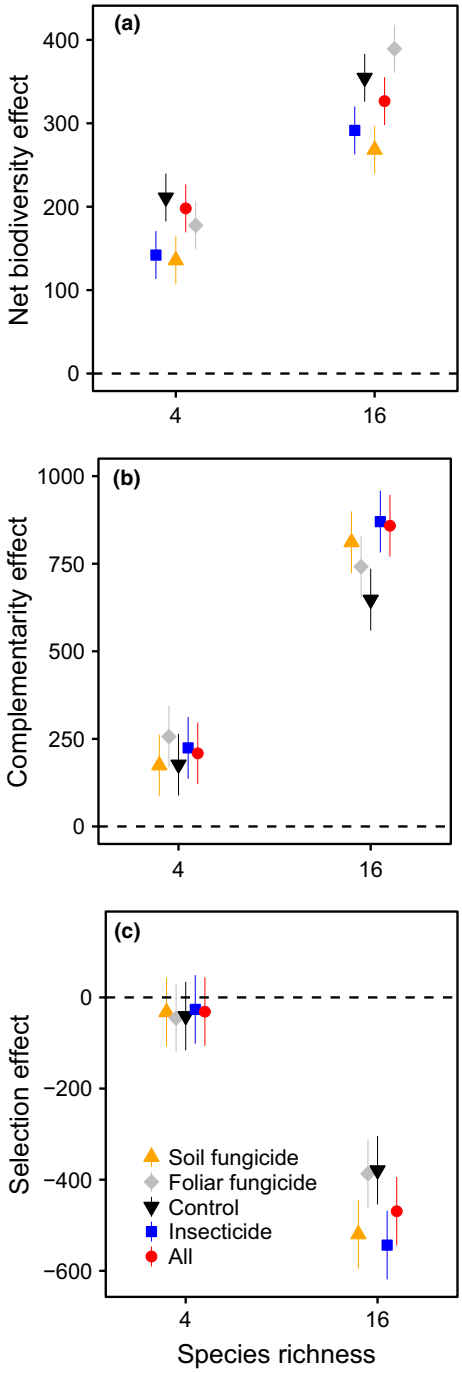

Figure 3 Effects of heterotroph removal on the net biodiversity, complementarity, and selection effects along an experimental diversity gradient. Heterotroph removal treatments were Control (black triangles), Foliar Fungicide (grey diamonds), Soil Fungicide (gold triangles), Insecticide (blue squares), or All Treatments in Combination (All; red circles). Error bars show 1 standard error around treatment means.

increased herbivorous arthropod mass on plant biomass across the plant diversity gradient outweighs the concurrent increase in abundance and diversity of predators and parasitoids.

Our results are concordant with a meta-analysis of insecticide studies that found an overall positive effect of insecticides on plant biomass (Coupe & Cahill 2003). While there are many arthropods that are beneficial to plants, the results suggest that the effects of positive plant–arthropod interactions are much less important than the direct removal of plant biomass by arthropod herbivores. Coupe & Cahill (2003) also found that the proportional effect of insecticide on plant productivity was unaffected by the plant richness in each study. While these studies did not manipulate diversity experimentally, the result aligns with our finding that insecticide application induces a constant proportional increase in plant biomass accumulation along the experimental diversity gradient.

The abundance of some fungal pathogen species has been shown to decline with increasing plant diversity (e.g. Mitchell *et al.* 2002; Keesing *et al.* 2006), including the experimental biodiversity plots where this study was conducted (Mitchell *et al.* 2002). However, studies showing dilution effects on pathogen prevalence or abundance usually focus on a single group of pathogens (e.g. foliar rust fungi) and typically do not measure ecosystem level impacts of these plant–consumer interactions. Our results suggest that while the dilution effect may reduce the prevalence some pathogen species at higher plant diversity, this may not translate to reduced impacts of all pathogens on ecosystem level processes such as primary productivity.

In contrast to our findings from this long-term experiment, previous work linking soil fungal abundance to plant biomass production in shorter term experiments (< 5 year old plant communities) found the effects of soil fungi on biomass were greatest in low-diversity plant communities, resulting in a flattening of the productivity diversity relationship (Maron *et al.* 2011; Schnitzer *et al.* 2011). Experiment duration may represent a key difference in the direction and magnitude of consumer effects in these studies (our study had 16–19 year old plant communities and 3–6 years of fungicide treatments). Diversity–productivity responses and mechanisms change through time (Fargione *et al.* 2007; Reich *et al.* 2012), and it is possible that soil fungi may play different roles as plant communities mature. Soil microbial communities can turn over rapidly in both space and time (Fierer *et al.* 2010; Will *et al.* 2010), and the impacts of soil fungi along diversity gradients can vary with the direction and strength of plant-soil feedbacks (Kulmatiski *et al.* 2012). Thus, the importance of soil fungi in determining plant productivity may depend on microbial composition and consequently be variable and context dependent.

While we found a net negative effect of the heterotrophic food web on plant productivity, the performance of most plants arises from the net effect of both positive and negative interactions (Morris *et al.* 2007). A deeper understanding would emerge from studies that specifically disentangle the balance between positive and negative interactions among heterotrophic groups along diversity gradients. For example, the abundance of different guilds of arthropods (e.g. herbivores, pollinators, predators and parasitoids) shift at different rates along experimental gradients in plant diversity, likely resulting in a shifting balance of positive and negative interactions (Haddad *et al.* 2009; Scherber *et al.* 2010; Hertzog *et al.* 2016).

The work presented here was conducted along experimental plant diversity gradients, and this may cause discrepancies

from what might occur along natural diversity gradients. For example, the experimental layout has created a mosaic of plant diversities within single field, and it is likely that there is movement of arthropods and fungi between adjacent plots. High rates of dispersal among plots would tend to obscure treatment differences making our tests relatively conservative. It also is possible that experimental communities respond differently than naturally assembled communities. Recent work has demonstrated that the positive effect of diversity on productivity found in experimental communities is an important factor along natural diversity gradients (Grace *et al*. 2016), and it would be informative to determine if the removal of consumers has comparable effects along diversity gradients in experimental and natural communities.

Interpretation of experiments using field applications of pesticides can be difficult due to possible effects of the treatments on plant growth that are due to factors other than reductions in the target organisms (e.g. foliar fungi, soil fungi or arthropod abundance). For example, pesticides might alter soil nutrient supplies by changing the composition of the soil biota (Bromilow *et al*. 1996; Johnsen *et al*. 2001; Bunemann *et al*. 2006). Our greenhouse experiment provided no evidence of these types of direct effects on plant growth; however, it is conceivable that the increased biomass resulting from pesticide application in our field plots may have reflected a fertilization effect of the pesticides rather than a reduction in fungi or arthropod abundance. Several lines of evidence suggest that the effect we have quantified is not a result of altered soil fertility. First, the effects of the pesticide removal on biomass (+44%) is much greater than is observed from direct fertilization (4 g N m^{-2} $year^{-1}$) in an adjacent experiment which manipulates both diversity and fertilization (+2 to +25%) (Reich *et al*. 2001), and it is unlikely that an indirect effect of the pesticides on soil fertility would be greater than direct fertilization. Second, the direct proportional fertilization effects reported in this adjacent experiment varied over tenfold across diversity treatments from 2% in the 1 species plots to 25% in the 16 species plots (Reich *et al*. 2001). In contrast, we found a constant proportional increase in biomass across the same diversity gradient of 1–16 species. Finally, increasing nutrient supply rates would generally increase plant tissue nutrient concentration (Bracken *et al*. 2015); however, this did not occur in this experiment (Borer *et al*. 2015).

The repeated demonstration that primary production increases with plant diversity has been one of the strongest direct lines of evidence of the value of diverse, intact communities to human well-being, leading to myriad analyses testing and contesting the mechanisms underlying this effect (Hector & Bagchi 2007; Isbell *et al*. 2011). At the core of most of these services and ecological mechanisms is the quantification of primary productivity (Hector & Bagchi 2007; Isbell *et al*. 2011). Here, we show that, far from being overplayed, the past experimental assessments of the impact of plant biodiversity on ecosystem processes likely have been conservative and biased downwards because the studies did not account for the portion of primary productivity that was transferred into the heterotrophic food web. The current results highlight the need for a multi-trophic perspective in theoretical and empirical studies examining the relationship between biodiversity and ecosystem functioning (Thebault & Loreau 2003; Duffy *et al*. 2007). In addition, our results suggest that the ongoing human-driven global simplification of ecosystems may have even greater effects on ecosystem functioning than previously thought.

AUTHORSHIP

All authors contributed to writing the paper. EWS, LK, ETB, RM and DT coordinated the experiments and data collection. EWS and YH analysed the data.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.b5b77.

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REFERENCES

Berendse, F. (1982). Competition between plant-populations with different root depths III. Field experiments. *Oecologia*, 53, 50–55.

Borer, E.T., Seabloom, E.W. & Tilman, D. (2012). Plant diversity controls arthropod biomass and temporal stability. *Ecol. Lett*., 15, 1457–1464.

Borer, E.T., Seabloom, E.W., Gruner, D.S., Harpole, W.S., Hillebrand, H., Lind, E.M. *et al*. (2014). Herbivores and nutrients control grassland plant diversity via light limitation. *Nature*, 508, 517–520.

Borer, E.T., Lind, E.M., Ogdahl, E.J., Seabloom, E.W., Tilman, D., Montgomery, R.A. *et al*. (2015). Food-web composition and plant diversity control foliar nutrient content and stoichiometry. *J. Ecol*., 103, 1432–1441.

Bracken, M.E.S., Hillebrand, H., Borer, E.T., Seabloom, E.W., Cebrian, J., Cleland, E.E. *et al*. (2015). Signatures of nutrient limitation and co-limitation: responses of autotroph internal nutrient concentrations to nitrogen and phosphorus additions. *Oikos*, 124, 113–121.

Bromilow, R.H., Evans, A.A., Nicholls, P.H., Todd, A.D. & Briggs, G.G. (1996). The effect on soil fertility of repeated applications of pesticides over 20 years. *Pestic. Sci*., 48, 63–72.

Bruno, J.F., Boyer, K.E., Duffy, J.E. & Lee, S.C. (2008). Relative and interactive effects of plant and grazer richness in a benthic marine community. *Ecology*, 89, 2518–2528.

Bunemann, E.K., Schwenke, G.D. & Van Zwieten, L. (2006). Impact of agricultural inputs on soil organisms - a review. *Aust. J. Soil Res*., 44, 379–406.

Clark, C.M. & Tilman, D. (2008). Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature*, 451, 712–715.

Coupe, M.D. & Cahill, J.F. (2003). Effects of insects on primary production in temperate herbaceous communities: a meta-analysis. *Ecol. Entomol*., 28, 511–521.

Craine, J.M., Tilman, D., Wedin, D., Reich, P., Tjoelker, M. & Knops, J. (2002). Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. *Funct. Ecol*., 16, 563–574.

van Der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T. *et al*. (1998). Mycorrhizal fungal

diversity determines plant biodiversity, ecosystem variability and productivity. *Nature (London)*, 396, 69–72.
Duffy, J.E., Cardinale, B.J., France, K.E., McIntyre, P.B., Thebault, E. & Loreau, M. (2007). The functional role of biodiversity in ecosystems: incorporating trophic complexity. *Ecol. Lett.*, 10, 522–538.
Durrant, W.E. & Dong, X. (2004). Systemic acquired resistance. *Annu. Rev. Phytopathol.*, 42, 185–209.
Fargione, J. & Tilman, D. (2005). Niche differences in phenology and rooting depth promote coexistence with a dominant C-4 bunchgrass. *Oecologia*, 143, 598–606.
Fargione, J., Tilman, D., Dybzinski, R., Lambers, J.H.R., Clark, C., Harpole, W.S. *et al.* (2007). From selection to complementarity: shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. *Proc. R. Soc. B Biol. Sci.*, 274, 871–876.
von Felten, S., Hector, A., Buchmann, N., Niklaus, P.A., Schmid, B. & Scherer-Lorenzen, M. (2009). Belowground nitrogen partitioning in experimental grassland plant communities of varying species richness. *Ecology*, 90, 1389–1399.
Fierer, N., Nemergut, D., Knight, R. & Craine, J.M. (2010). Changes through time: integrating microorganisms into the study of succession. *Res. Microbiol.*, 161, 635–642.
Fornara, D.A. & Tilman, D. (2009). Ecological mechanisms associated with the positive diversity-productivity relationship in an N-limited grassland. *Ecology*, 90, 408–418.
Grace, J.B., Anderson, T.M., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S. *et al.* (2016). Integrative modelling reveals mechanisms linking productivity and plant species richness. *Nature*, 529, 390-+.
Haddad, N.M., Tilman, D., Haarstad, J., Ritchie, M. & Knops, J.M.H. (2001). Contrasting effects of plant richness and composition on insect communities: a field experiment. *Am. Nat.*, 158, 17–35.
Haddad, N.M., Crutsinger, G.M., Gross, K., Haarstad, J., Knops, J.M.H. & Tilman, D. (2009). Plant species loss decreases arthropod diversity and shifts trophic structure. *Ecol. Lett.*, 12, 1029–1039.
Hector, A. & Bagchi, R. (2007). Biodiversity and ecosystem multifunctionality. *Nature*, 448, 188–U186.
Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G. *et al.* (1999). Plant diversity and productivity experiments in European grasslands. *Science*, 286, 1123–1127.
Hertzog, L.R., Meyer, S.T., Weisser, W.W. & Ebeling, A. (2016). Experimental manipulation of grassland plant diversity induces complex shifts in aboveground arthropod diversity. *PLoS ONE*, 11, 1–16.
Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W.S., Reich, P.B. *et al.* (2011). High plant diversity is needed to maintain ecosystem services. *Nature*, 477, 199–U196.
Johnsen, K., Jacobsen, C.S., Torsvik, V. & Sorensen, J. (2001). Pesticide effects on bacterial diversity in agricultural soils - a review. *Biol. Fertil. Soils*, 33, 443–453.
Keesing, F., Holt, R.D. & Ostfeld, R.S. (2006). Effects of species diversity on disease risk. *Ecol. Lett.*, 9, 485–498.
Koricheva, J., Mulder, C.P.H., Schmid, B., Joshi, J. & Huss-Danell, K. (2000). Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grasslands. *Oecologia*, 125, 271–282.
Kulmatiski, A., Beard, K.H. & Heavilin, J. (2012). Plant-soil feedbacks provide an additional explanation for diversity-productivity relationships. *Proc. R. Soc. B Biol. Sci.*, 279, 3020–3026.
Lind, E.M., Borer, E., Seabloom, E., Adler, P., Bakker, J.D., Blumenthal, D.M. *et al.* (2013). Life-history constraints in grassland plant species: a growth-defence trade-off is the norm. *Ecol. Lett.*, 16, 513–521.
Loreau, M. & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, 412, 72–76.
Maron, J.L., Marler, M., Klironomos, J.N. & Cleveland, C.C. (2011). Soil fungal pathogens and the relationship between plant diversity and productivity. *Ecol. Lett.*, 14, 36–41.
McKane, R.B., Grigal, D.F. & Russelle, M.P. (1990). Spatiotemporal differences in N-15 uptake and the organization of an old-field plant community. *Ecology*, 71, 1126–1132.
Mitchell, C.E., Tilman, D. & Groth, J.V. (2002). Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. *Ecology*, 83, 1713–1726.
Morris, W.F., Hufbauer, R.A., Agrawal, A.A., Bever, J.D., Borowicz, V.A., Gilbert, G.S. *et al.* (2007). Direct and interactive effects of enemies and mutualists on plant performance: a meta-analysis. *Ecology*, 88, 1021–1029.
Mueller, K.E., Tilman, D., Fornara, D.A. & Hobbie, S.E. (2013). Root depth distribution and the diversity-productivity relationship in a long-term grassland experiment. *Ecology*, 94, 787–793.
Naeem, S., Bunker, D.E., Hector, A., Loreau, M. & Perrings, C. (2010). *Biodiversity, Ecosystem Functioning, & Human Wellbeing: An Ecological and Economic Perspective*. Oxford University Press, Oxford, UK.
Naeem, S., Duffy, J.E. & Zavaleta, E. (2012). The Functions of Biological Diversity in an Age of Extinction. *Science*, 336, 1401–1406.
Reich, P.B., Knops, J., Tilman, D., Craine, J., Ellsworth, D., Tjoelker, M. *et al.* (2001). Plant diversity enhances ecosystem responses to elevated CO2 and nitrogen deposition. *Nature*, 410, 809–812.
Reich, P.B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S.E., Flynn, D.F.B. *et al.* (2012). Impacts of biodiversity loss escalate through time as redundancy fades. *Science*, 336, 589–592.
Roscher, C., Schumacher, J., Gubsch, M., Lipowsky, A., Weigelt, A., Buchmann, N. *et al.* (2012). Using plant functional traits to explain diversity-productivity relationships. *PLoS ONE*, 7, 1–11.
Scherber, C., Eisenhauer, N., Weisser, W.W., Schmid, B., Voigt, W., Fischer, M. *et al.* (2010). Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature*, 468, 553–556.
Schnitzer, S.A., Klironomos, J.N., HilleRisLambers, J., Kinkel, L.L., Reich, P.B., Xiao, K. *et al.* (2011). Soil microbes drive the classic plant diversity-productivity pattern. *Ecology*, 92, 296–303.
Thebault, E. & Loreau, M. (2003). Food-web constraints on biodiversity-ecosystem functioning relationships. *Proc. Natl Acad. Sci. USA*, 100, 14949–14954.
Thebault, E. & Loreau, M. (2006). The relationship between biodiversity and ecosystem functioning in food webs. *Ecol. Res.*, 21, 17–25.
Tilman, D., Wedin, D. & Knops, J. (1996). Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature (London)*, 379, 718–720.
Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T. & Lehman, C. (2001). Diversity and productivity in a long-term grassland experiment. *Science*, 294, 843–845.
Tilman, D., Reich, P.B. & Knops, J.M.H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, 441, 629–632.
Tilman, D., Reich, P.B. & Isbell, F. (2012). Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. *Proc. Natl Acad. Sci. USA*, 109, 10394–10397.
Will, C., Thurmer, A., Wollherr, A., Nacke, H., Herold, N., Schrumpf, M. *et al.* (2010). Horizon-specific bacterial community composition of German grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Appl. Environ. Microbiol.*, 76, 6751–6759.

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