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Does herbivory really suppress mycorrhiza? A meta-analysis

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Summary

1. In managed and natural ecosystems, herbivory can have a major impact on plant growth. Defoliation and shoot removal reduce the photosynthetic capacity of plants and are also thought to reduce allocation of carbon to roots and mycorrhizal fungi. However, evidence supporting this assumption remains equivocal.

2. We conducted a meta-analysis of 99 experiments, from 33 publications, measuring arbuscular and ectomycorrhizal colonization of roots after real or simulated herbivory removed leaves or shoots. We also explored how effects were moderated by the type of mycorrhiza, fertilization, experimental setting (laboratory or field), treatment (real herbivory of shoots, real herbivory of leaves, simulated herbivory of shoots, simulated herbivory of leaves), type of host plant, duration of the experiment and year of publication.

3. Overall, herbivory reduced mycorrhizal colonization by about 3 percentage points. Treatment, host plant type and year of publication were the only significant moderators, with real or simulated herbivory of leaves and real herbivory of shoots suppressing colonization, while simulated removal of shoots tended to increase colonization. Herbivory reduced colonization of perennial grasses and deciduous trees by about 4 and 8 percentage points, respectively. Mycorrhizal colonization of annual crops was reduced by about 12 percentage points, although this was not significantly different from zero. Mycorrhizal colonization of perennial forbs and evergreen trees was also unaffected by herbivory, and colonization of mixtures of perennial grasses and forbs increased by about 15 percentage points following herbivory. Effect size increased with year of publication, likely due to shifts in experimental designs towards systems more likely to show positive effects of herbivory on mycorrhiza.

4. *Synthesis*. Our results challenge the carbon-limitation hypothesis and suggest that herbivory, real or simulated, reduces mycorrhizal colonization by biologically meaningful amounts only in a limited subset of systems.

Key-words: arbuscular myccorrhyiza, clipping, defoliation, ectomycorrhiza, grazing, insect herbivory, land use, meta-analysis, mowing

Introduction

Removal of above-ground biomass, whether by insect or mammalian herbivory, or by clipping or mowing, alters the carbon budget of the plant by removing photosynthetic tissue, forcing physiological changes in the plant. Despite the need to re-grow above-ground tissue, carbon allocation to roots often increases immediately after herbivory or clipping, while allocation to shoots decreases (Dyer *et al*. 1991; Holland, Cheng & Crossley 1996). Much of this carbon is maintained in readily accessible pools within the root or released into the rhizosphere as root exudates (Dyer *et al*. 1991; Holland, Cheng & Crossley 1996), with concurrent increases in the abundance of bacteria, enchytraeids and microbe-feeding nematodes (Holland 1995; Mawdsley & Bardgett 1996; Mikola *et al*. 2001). Root mutualists such as rhizobia and mycorrhizal fungi, which must obtain carbon from their plant hosts, would not benefit directly from increased carbon exudation rates (Smith & Read 2008), but could still benefit from increased carbon flux to roots (Waters & Borowicz 1994). After these initial increases in carbon allocation to roots, over the long-term herbivory and clipping tend to reduce carbon allocation to roots (Bardgett, Wardle & Yeates 1998). At this point, the carbon-limitation hypothesis posits that growth of mycorrhizal fungi will be reduced because carbon will be preferentially allocated to other plant or soil pools (Gehring & Whitham 2002).

Mycorrhizal fungi make important contributions to plant growth by increasing nutrient uptake and improving water

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relationships and resistance to pathogens (Newsham, Fitter & Watkinson 1995; Azcón-Aguilar & Barea 1996; Smith & Read 2008); reduction in fungal growth may thus be especially detrimental following extensive loss of above-ground tissues. Mycorrhizal effects on insect herbivores depend on the identity of the fungus and herbivore, so although reductions in fungal colonization could allow greater herbivory by some insects (polyphagous chewers and mesophyll-feeding sucking insects), growth of others that benefit from mycorrhizal colonization (mono- and oligophagous chewers and phloem feeders) would be slowed (Koricheva, Gange & Jones 2009). Mycorrhizal fungi also perform important ecosystem services such as soil aggregation, nutrient cycling and carbon sequestration (Rillig 2004; Rillig & Mummey 2006; Smith & Read 2008). Using a vote counting approach to summarize the literature, Gehring & Whitham (1994) found that mycorrhizal colonization declined after herbivory in 62.2% of the 37 plant species studied, while colonization increased in only 5.4% of species. Colonization was either unaffected or inconsistently affected in 32.4% of the plant species studied. An updated review in 2002, which included an additional five plant species, found very similar patterns, with colonization reduced after herbivory in 64.3% of plant species studied. Mycorrhizal colonization increased after herbivory in only 4.8% of plant species, and colonization was unaffected or inconsistently affected by herbivory in the remaining 30.9% of plant species (Gehring & Whitham 2002). The carbon-limitation hypothesis is widely invoked to explain these results (Gehring & Whitham 1994, 2002; Wardle 2002; Wardle *et al*. 2004), but despite the intuitive logic of the hypothesis, positive effects of herbivory on mycorrhizal growth continue to be reported.

The variability in responses may be due to the type and extent of herbivory or clipping, the duration of the experiment, the type of mycorrhiza or plant studied, or other as yet unrecognized factors (Gehring & Whitham 2002; Gehring & Bennett 2009). For example, insect herbivory and grazing may induce costly defence responses that limit regrowth more than mechanical defoliation by mowing or clipping. Also, insect herbivory often destroys foliage while leaving apical meristems intact, but grazing can also remove apical meristems. Removal of small amounts of tissue could induce compensatory growth responses in plants especially dependent on mycorrhizas, which could increase root colonization rates. The mycorrhizal response to above-ground tissue loss may be short term, with colonization levels returning to normal as new biomass is produced. Ecto and arbuscular mycorrhiza have distinct physiology and may respond differently to tissue loss. In particular, some ectomycorrhizal fungi (ECM) can act saprophytically so their carbon budget is not tied to the host plant as closely as that of arbuscular mycorrhizal fungi (AMF) (Smith & Read 2008). Annual crop species, perennial herbaceous plants, and woody species have very different growth strategies and can respond differently to tissue loss (Obeso 1993; Hawkes & Sullivan 2001). Annual plants may use stored resources for reproduction, while perennial grasses may invest in compensatory shoot growth. Woody species often have extensive carbon reserves below ground and can rapidly replace lost tissue, even after losing large amounts of above-ground biomass (Kosola *et al*. 2004). These strategies leave different amounts of energy available to the root system, so mycorrhizal fungi would be expected to respond differently to tissue loss as well. Finally, soil fertility mediates many aspects of mycorrhizal interactions (Smith & Read 2008) and may also be important following herbivory. We conducted a meta-analysis in order to summarize quantitatively published reports on the sensitivity of mycorrhizal fungi to herbivory or clipping of their plant hosts, and to determine how that sensitivity is moderated by the type of mycorrhiza, fertilization, treatment, experimental setting, host plant type, duration of the experiment, year of publication, and frequency and degree of defoliation.

Materials and methods

We began with a literature search for studies measuring effects of real or simulated herbivory, removing leaves and/or shoots, on mycorrhizas (AMF and ECM), with the following search string, [mycorrh* and (mow* or clip* or defoliat* or cut* or insect* or graz* or feed* or brows* or pastur* or herbivor* or cattle or cow* or sheep or horse* or rabbi*)]. The search was conducted on 24 March 2009, using the Web of Science Citation Index Expanded data base and including all articles published since 1965. The initial search returned 951 publications, and we scanned these abstracts to identify studies where mycorrhizal abundance was quantified after removal of above-ground biomass. Of these papers, we found 33 that reported percentage root length colonized in control and treatment plants, along with sample sizes (N) and either standard deviation (SD) or standard error (SE). When necessary, this information was retrieved from digitized graphs with GraphClick v3.0 (Arizona Software, Neuchatel, Switzerland). When SE was reported, we calculated SD as: $SD = SE \times \mathrm{sqrt}(N)$. We assumed that unidentified error bars represented SE.

When one publication reported effects in more than one system, we treated each as a separate experiment, resulting in 99 separate experiments. In order to be considered a separate system, experiments had to differ in at least one of the following factors: study site, plant species, AMF species, fertilization treatment (yes or no) and method or degree of defoliation. Where multiple experiments differed only in treatment duration, only the longest time period was considered. For all experiments, we recorded the duration of the experiment as the time between the first herbivory event and the harvest, and whether or not chemical fertilizers were used during the experiment. When fertilization was not explicitly mentioned, we assumed that no fertilizer was applied. When reported, we also recorded the number of cuts per year for mowing and clipping experiments. Stocking intensity and soil type were not consistently reported so we could not include them in the model, and we did not have sufficient replicates to include species of grazing animal.

All analyses were conducted in R (R Development Core Team 2008), with the 'meta' package (Schwarzer 2009) and the 'MiMa' function (Viechtbauer 2006).We performed a continuous meta-analysis using a mixed-effects model where the overall analysis was performed with a random-effects model using the DerSimonian–Laird method, and comparisons across subgroups during tests of moderators were performed using a fixed-effects model (Borenstein *et al*. 2009). We used the weighted mean difference (WMD = treatment mean − control mean) as the effect size for each experiment and pooled experiments by inverse variance weighting (Borenstein *et al*. 2009). Since we only included experiments reporting percentage colonization of roots, which was always reported on a scale from 0% to

100%, we did not need to correct for different reporting scales by using Hedge's g or the response ratio (Borenstein *et al.* 2009). This simplifies interpretation of results since the WMD remains in the original units of percentage colonization. We used a Box–Cox transformation to correct non-normality of WMDs before analysis, but we report back-transformed WMDs with 95% confidence intervals. We tested for publication bias with the 'metabias' function using Kendall's rank correlation between standardized treatment estimates and variance estimates of estimated treatment effects, where the test statistic Z is a modified Kendall's tau (Begg & Mazumdar 1994).

We performed chi-squared contingency table tests to assess independence of moderators, and then used the MiMa function with restricted maximum-likelihood estimation to test for effects of moderators. We analysed all the experiments together to test for effects of type of mycorrhiza (AMF or ECM), fertilization (yes or no), treatment (real herbivory of leaves, real herbivory of shoots, simulated herbivory of leaves, simulated herbivory of shoots), setting (field or laboratory), plant type (annual crop, perennial grass, perennial forb, deciduous tree, evergreen tree, or a mix of perennial grasses and forbs), experiment duration and year of publication. We also considered the number of cuts per year in experiments where plants were clipped or mowed, and the percentage of defoliation in experiments using insect herbivores to defoliate plants. In addition, we performed meta-analyses with the subsets of data published before 1994 and 2002 in order to compare our results with those found in previous reviews using a vote counting approach (Gehring & Whitham 1994, 2002). We report the overall WMD, and total between experiments heterogeneity (Q) for the overall meta-analysis. For tests of moderators, we report WMD of each level of each moderator, residual heterogeneity (Q_E), and between groups heterogeneity (Q_{ME}).

In order to deal with non-independence of moderators, we analysed effects of moderators within the largest subgroups of data separately. Within experiments using perennial grasses, we tested the effects of fertilization, treatment and setting. All experiments using grasses also used AMF, so type of mycorrhiza was not considered. Within the subset of experiments using AMF, we analysed the moderator plant type. Sample sizes in other subgroups were too small to allow meaningful tests of moderators.

In order to determine if experimental designs were shifting over time, we performed correlation analyses between the levels of each moderator and the year of publication. We began by sorting the levels of each moderator by mean year of publication to determine in which order to place the levels of each moderator. Then, we assigned all the experiments in the level with the earliest mean year of publication a value of 1, all the experiments in the level with the second earliest year of publication a value of 2, and so on so that all the experiments in the level with the most recent mean year of publication had the highest value. We then correlated these values with the year of publication to determine whether or not use of moderators was changing over time. Despite sorting groups of experiments by their mean year of publication in the initial step, this approach will not create correlations if use of moderators did not change over time.

Results

There was no evidence of publication bias ($Z = 0.0181$, $P = 0.9855$), showing that insignificant results were as likely to be published as significant results. Overall, herbivory or clipping reduced root colonization by mycorrhizal fungi by about 3 percentage points [WMD = −2.96 (−5.48; −0.49); $Z = -2.3683$, $P = 0.0179$]. There was significant heterogeneity among experiments ($Q = 1\,122\,352.36$, d.f. = 98, $P < 0.0001$; see Table S1 in Supporting Information), indicating that other experimental treatments, or moderators, may have influenced results. The type of mycorrhiza, fertilization, setting and duration of the experiment did not affect effect sizes (Table 1). In mowed or clipped sites, the number of cuts per year had no effect on effect sizes ($N = 60$, $Q_E = 10\,026.56$, $Q_{ME} = 1.5945$, d.f. = 1, $P = 0.2067$). Percentage defoliation of insect-defoliated plants also had no effect on effect sizes

Table 1. Group mean effect sizes (WMD) with statistical significance of moderators

Moderator	Groups	WMD	95% CI	Number of studies	Q_E[a]	Q_{ME}[b]	P-value
Type of mycorrhizas	AMF	−2.13	−4.89; 0.55	82	1 114 883	1.6938	0.1931
	ECM	−7.03	−13.42; −1.05	17			
Fertilization	Yes	−3.17	−8.58; 1.97	23	1 066 843	0.0071	0.9327
	No	−2.89	−5.72; −0.14	76			
Treatment	Simulated – leaves	−5.52	−9.98; −1.25	35	992 676	4.3517	0.0370
	Simulated – shoots	3.65	−0.36; 7.52	28			
	Real – leaves	−4.46	−8.89; −0.21	30			
	Real – shoots	−13.07	−19.86; −6.82	6			
Setting	Field	−2.69	−5.64; 0.18	66	1 073 323	0.0700	0.7913
	Laboratory	−3.48	−8.61; 1.39	33			
Plants	Annual crop	−12.44	−32.19; 3.26	5	1 108 859	5.5737	0.0182
	Grass – perennial	−3.96	−7.94; −0.13	42			
	Forb – perennial	−1.16	−5.43; 2.95	28			
	Deciduous	−8.39	−14.73; −2.47	7			
	Evergreen	−5.04	−13.62; 2.85	12			
	Mix	15.14	9.73; 20.34	5			
Duration					1 122 167	0.0342	0.8533
Year of publication					1 065 246	19.7262	< 0.0001

[a]Residual heterogeneity.
[b]Between groups heterogeneity.

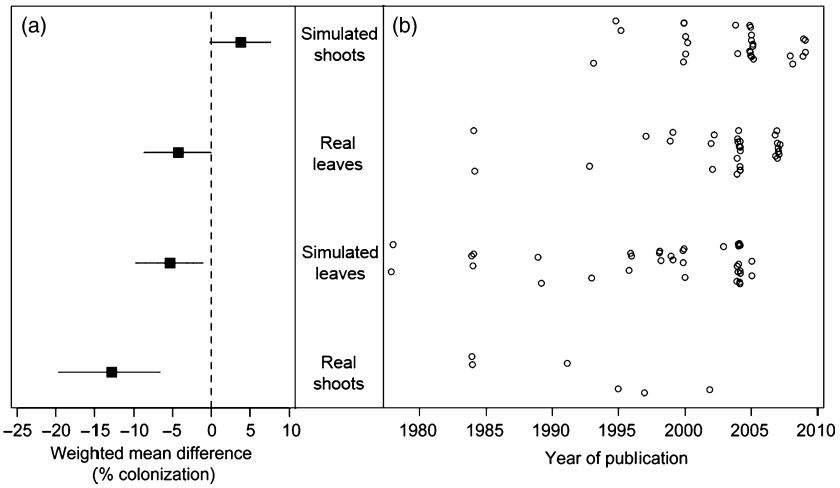

Fig. 1. (a) Effect sizes (mean ± 95% CI) for each herbivory application method. (b) Correlation between herbivory application method and year of publication. Treatments are sorted with the earliest mean date of publication appearing at the bottom of the figure. Points are jittered on both axes to improve visibility of overlapping points. Simulated shoots, simulated herbivory of shoots; real leaves, real herbivory of leaves; simulated leaves, simulated herbivory of leaves; real shoots, real herbivory of shoots.

(N = 18, Q_E = 657.08, Q_{ME} = 0.0.6204, d.f. = 1, P = 0.4309). The only significant moderators were year of publication, treatment and host plant type. Real herbivory of shoots led to the greatest reduction in mycorrhizal colonization (13 percentage points). Real or simulated herbivory of leaves reduced colonization by about 5 percentage points, and simulated herbivory of shoots tended to enhance colonization (Table 1, Fig. 1a). Annual crops suffered the greatest inhibition of mycorrhizal colonization after herbivory (13 percentage points), followed by deciduous trees with a reduction of about 8 percentage points. Perennial grasses showed a reduction of colonization of almost 4 percentage points. Herbivory had no effect on colonization of evergreen trees and increased colonization in mixes of perennial grasses and forbs (Table 1, Fig. 2a).

The effect size was positively correlated with the year of publication (Fig. 3, Table 2). We performed correlations between year and all the moderators to determine which moderators, if any, were changing with year and could explain the observed pattern. Experimental setting has changed over time (Table 2), from laboratory to field experiments. Despite the change, this moderator did not alter the effect of herbivory, so this change cannot explain the correlation between effect size and year of publication. However, the type of plant used in experiments and the treatment method have also changed over time (Table 2) and significantly affected the response to defoliation (Table 1).

After the early attention paid to annual crops, evergreen trees received fairly even study from 1991 to 2003. Perennial grasses and forbs have been used since 1984 and have been more widely used since 1999. Deciduous trees have been less common study subjects, and only between 1997 and 2004. Beginning in 2004, more realistic mixed communities made up of common grassland plants have been incorporated into experimental designs. This shift in the type of plant studied over time generally parallels the increase in mean effect sizes of

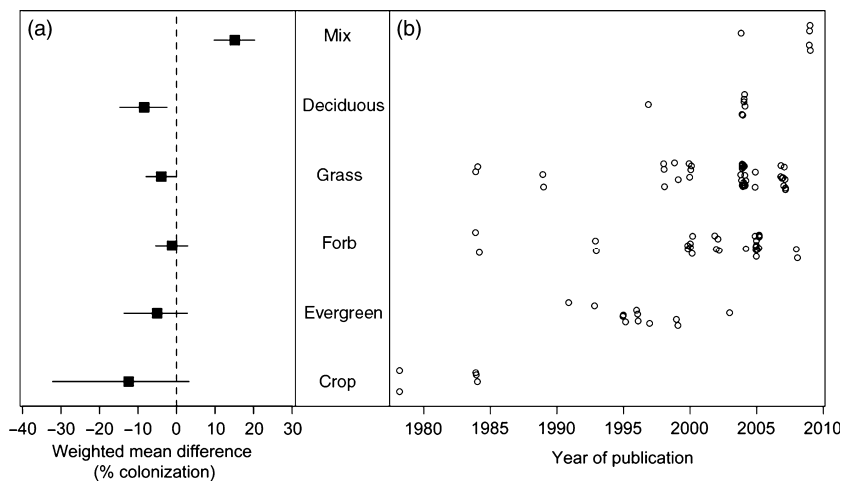

Fig. 2. (a) Effect sizes (mean ± 95% CI) for each plant type. (b) Correlation between plant type and year of publication. Plant types are sorted with the earliest mean date of publication appearing at the bottom of the figure. Points are jittered on both axes to improve visibility of overlapping points.

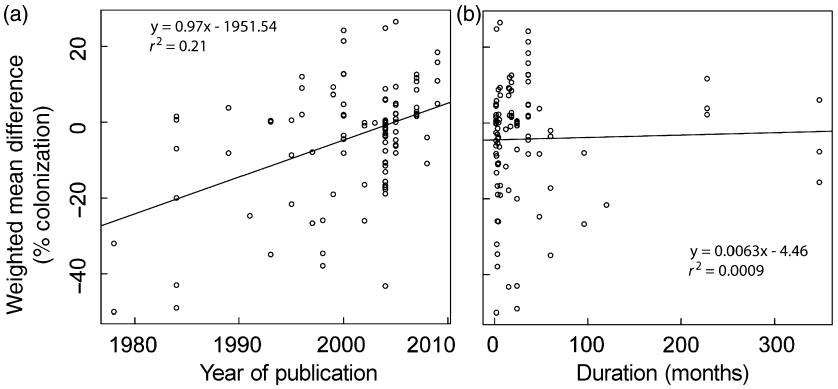

Fig. 3. Correlations between weighted mean difference and (a) year of publication, and (b) duration of experiment, which is the time from first herbivory treatment to harvest. Trend lines are from mixed-effects meta-analytic models.

each plant type and probably contributed to the correlation between year of publication and effect size (Fig. 2).

The temporal trend in effect size may also be partly explained by a shift in the type of treatment applied in herbivory studies. Only six studies, which were published between 1984 and 2002, used live herbivores (insects or mammals), which removed or damaged apical meristems. The use of simulated removal of foliage as a treatment began in 1978 and has increased in recent years, with the most recent studies using this treatment published in 2005. Insect and mammalian herbivory have been studied since 1984, with most studies using this technique published between 2004 and 2007. The first study simulating removal of shoots, including apical meristems, was published in 1993, and most studies using this treatment method were published after 2005. The reduction in colonization following each of these treatments parallels the shift in application rate of each treatment (Fig. 1), probably contributing to the correlation between year of publication and effect size.

Chi-squared contingency table analysis showed that most combinations of moderators were not independent (Table 3), indicating that effects of one moderator cannot be completely separated from effects of other moderators. We tested for effects of fertilization, treatment and setting within the subset of experiments using perennial grasses and found that treatment was no longer significant (Table 4). However, none of the experiments with grasses removed shoots, and the largest differences in the complete data set were between the experiments removing leaves and those removing shoots. Setting was a significant moderator in experiments using perennial grasses, with results in laboratory experiments giving strongly negative results (reduction of *c.* 13 percentage points) while field experiments showed no effect of herbivory. Within experiments using AMF, host plant type was a marginally significant moderator (Table 5). There were no experiments using evergreen trees and AMF and only two with deciduous trees. As with the complete data set, the largest differences were between annual crops and mixes of perennial grasses and forbs.

When including only the data available from before 1994, the mean effect size was much lower than when all the data were included [N = 15; WMD = −14.61 (−21.33; −8.43); Z = −4.8613, P < 0.0001]. The mean effect size of all the experiments published before 2002 was slightly lower than the

Table 2. Results of Pearson correlations between moderators and year of publication

d.f. = 97	*R*	*t*	*P*-value
Type of mycorrhiza[a]	0.1487	1.4808	0.1419
Fertilization[b]	0.1954	1.9627	0.0526
Treatment[c]	0.4333	4.7346	< 0.0001
Setting[d]	0.2095	2.1102	0.0374
Plant[e]	0.5757	6.9349	< 0.0001

[a]ECM = 1, AMF = 2.
[b]Yes = 1, no = 2.
[c]Real herbivory of shoots = 1, simulated herbivory of leaves = 2, real herbivory of leaves = 3, simulated herbivory of shoots = 4.
[d]Laboratory = 1, field = 2.
[e]Annual crop = 1, evergreen = 2, perennial forb = 3, perennial grass = 4, deciduous tree = 5, mix of perennial grasses and forbs = 6.

Table 3. Results of chi-squared contingency analysis for independence of moderators

	Myc[a]	Fert[b]	Treatment[c]	Setting[d]	Plants[e]
Myc	**2**[f]	2.39[g]	6.70	0.01	88.96
Fert	0.1222[h]	**2**	10.68	3.75	22.64
Treatment	0.0823	0.0136	**4**	15.09	85.33
Setting	0.9250	0.0530	0.0017	**2**	17.13
Plants	< 0.0001	0.0004	< 0.0001	0.0043	**6**

[a]Type of mycorrhiza (AMF or ECM).
[b]Fertilization (yes or no).
[c]Treatment (real herbivory of leaves, real herbivory of shoots, simulated herbivory of leaves, simulated herbivory of shoots).
[d]Setting of experiment (laboratory or field).
[e]Host plant type (annual crop, perennial grass, perennial forb, deciduous tree, evergreen tree, mix of perennial grasses and forbs).
[f]Number of levels of each moderator are shown in bold on the diagonal.
[g]Chi-squared statistics are shown above the diagonal.
[h]*P*-values are shown below the diagonal.

Table 4. Group mean effect sizes (WMD), with statistical significance of moderators, for the subset of experiments using perennial grasses

Moderator	Groups	WMD	95% CI	Number of studies	Q_E	Q_{ME}	*P*-value
Fertilization	Yes	−1.81	−13.23; 8.48	8	393 607	0.2435	0.6217
	No	−4.48	−8.07; −1.01	34			
Treatment	Simulated – leaves	−4.68	−10.23; 0.58	23	513 679	0.1356	0.7126
	Simulated – shoots	NA	NA	NA			
	Real – leaves	−3.09	−8.53; 2.07	19			
	Real – shoots	NA	NA	NA			
Setting	Field	−1.42	−5.40; 2.42	32	420 550	5.2102	0.0225
	Glasshouse	−12.61	−21.66; −4.48	10			

NA indicates that there were no experiments using perennial grasses and this moderator.
Q_E, residual heterogeneity; Q_{ME}, between groups heterogeneity.

Table 5. Group mean effect sizes (WMD), with statistical significance of moderators, for the subset of experiments using AMF

Moderator	Groups	WMD	95% CI	Number of studies	Q_E	Q_{ME}	*P*-value
Plants	Annual crop	−12.44	−32.19; 3.26	5	1 049 786	3.3524	0.0671
	Grass – perennial	−3.96	−7.94; −0.13	42			
	Forb – perennial	−1.16	−5.43; 2.95	28			
	Deciduous	0.04	−0.44; 0.52	2			
	Evergreen	NA	NA	NA			
	Mix	15.14	9.73; 20.34	5			

NA indicates that there were no experiments using AMF and this moderator.
Q_E, residual heterogeneity; Q_{ME}, between groups heterogeneity.

overall mean when all available data were included [$N = 40$; WMD = −6.19 (−10.78; −1.82); $Z = -2.8051$, $P = 0.0050$].

Discussion

The carbon-limitation hypothesis prediction of reduced mycorrhizal growth following removal of above-ground biomass was generally not supported. Overall, herbivory reduced colonization by about 3 percentage points, but this decrease is so small as to be not biologically meaningful. Treatment, host plant type and year of publication were the only significant moderators. The type of mycorrhiza used, fertilization, experimental setting and duration, and degree and frequency of herbivory all did not moderate the effect of herbivory on mycorrhizal colonization of roots.

We expected treatment to be an important moderator since removal of only foliage and removal of apical meristems induce distinct physiological responses in the plant. Real or simulated herbivory of leaves, and real herbivory of shoots, suppressed mycorrhizal colonization, while simulated herbivory of shoots had no effect, suggesting that when shoots are browsed, less foliage is consumed than when foliage alone is browsed. This would leave greater amounts of photosynthetic tissue on the plant, which could better support mycorrhizal fungi.

Also as expected, host plant type was an important moderator; with the most sensitive plant type being the short-lived annual crops. These plants have limited carbon reserves and may not be able to recover from extreme herbivory. However, this result was driven largely by two experiments from the same publication, using maize and tomato, where colonization was reduced by 32% and 50%, respectively (Daft & El-Giehmi 1978). Three experiments using soybeans, all from the same publication, found either no effect or a slightly positive effect of herbivory (Bayne, Brown & Bethlenfalvay 1984). With so few experiments reporting work with annual plants, it is clear that more research is needed before these results can be interpreted conclusively.

The gradual shift of research from the use of plant types and treatment methods showing strong negative effects to those showing strong positive effects explains the correlation of effect size with year of publication. The temporal trend of increasing effect sizes in more recent publications may also explain why our conclusion of minimal effects of defoliation on mycorrhizal colonization differ from that found by Gehring & Whitham (1994). When we analysed only the experiments published before 1994, we found a mean effect size almost four times lower than when all available data are included. The experiments we included in our meta-analysis do not overlap completely with those reviewed by Gehring and Whitham due to our more stringent selection criteria, but 60% of the experiments published before 1994 that we included reported negative effects, a value quite similar to that reported by Gehring & Whitham in 1994 (62%). As the rate of publication of experiments reporting positive effects increased, the overall mean effect size increased as well, and by 2002, the overall mean effect was similar to that found in our complete analysis, with only 52% of experiments reporting negative effects. This

contrasts with the value reported by Gehring & Whitham (2002) of 64%, but is very similar to our final meta-analysis where 53% of experiments reported negative effects. We analysed effects of multiple types of defoliation, while Gehring and Whitham focused on insect defoliation and attempts to mimic insect defoliation, and this may explain why a meta-analysis of experiments published before 2002 does not agree with the Gehring & Whitham (2002) review. Also, vote counting approaches do not take into account the magnitude of the effect, so a large proportion of studies reporting negative effects does not necessarily mean that the overall effect is large.

Type of mycorrhiza was not a significant moderator, but it was strongly confounded with host plant type, which was significant. Since many plant types only associate with one type of mycorrhiza, it may not be possible to separate the moderating effects of mycorrhiza and plant type. Instead, future research should focus on elucidating the response mechanisms operating in different host plant types.

We expected experimental duration to be an important moderator because carbon allocation patterns are thought to shift following herbivory. Increased allocation to roots begins within a matter of hours and can return to normal in less than a week (Dyer *et al*. 1991). Most of the experiments we analysed had durations on the order of months, and may have been too long to see any brief responses immediately following herbivory events.

Variation in mycorrhizal response to loss of above-ground biomass has been suggested to be due to the amount of tissue removed (Gehring & Whitham 2002; Gehring & Bennett 2009). More extensive tissue loss is predicted to lead to stronger effects on mycorrhizas because carbon will be more limiting. We quantified this in two separate tests, using the number of cuts per year in clipping experiments and the percentage of defoliation for insect herbivory experiments, but neither moderator was significant. In a meta-analysis of defoliation effects on growth of grasses, Ferraro & Oesterheld (2002) found that the percentage defoliation did not influence effect size, but effects were more severe as defoliation frequency increased. They also found stronger negative effects in fertilized sites, in contrast to our results finding no effect of fertilization. Focusing on perennial grasses, they found significant reductions in above-ground biomass after defoliation, but no significant effects on root biomass. This resilience of root biomass to defoliation may explain the lack of effect we observed on mycorrhizal colonization of grasses.

An additional concern is the application of simulated herbivory treatments in an attempt to mimic more complex defoliation events. For example, clipping is commonly applied in the laboratory with the intention of creating effects similar to those caused by grazing in the field. Grazing, however, also provides nutrients in the form of manure and soil compaction caused by the weight of the animals. Both of these factors may influence plant responses, but neither is included in most laboratory experiments meant to study effects of grazing. In the complete data set, we found no evidence that mycorrhizal responses depended on whether herbivory was real or simulated, suggesting that results of experiments applying clipping treatments in the laboratory to simulate effects of grazing or insect herbivory in the field may be valid. However, among experiments using only perennial grasses, results found in the laboratory were more strongly negative than results from field experiments, showing that the applicability of laboratory results to field situations can vary with host plant type.

Species composition of the mycorrhizal community could be altered by defoliation, even if absolute fungal abundance is unaffected. Growth of many mycorrhizal species does seem to be limited by defoliation, but other species that are able to cope with reduced carbohydrate input from the plant may actually thrive after defoliation of their host plants (Eom, Wilson & Hartnett 2001; Saito *et al*. 2004; Su & Guo 2007; Saravesi *et al*. 2008). These changes in species diversity may reflect changes in functional diversity of mycorrhizal communities as well, as suggested by Frank *et al*. (2003), who used soils from field sites with and without a history of grazing to inoculate plants in a glasshouse setting. Inocula from grazed sites led to greater increases in plant biomass than inocula from ungrazed sites, suggesting that defoliation can select for more beneficial AMF communities. However, this may have been an effect of the greater initial inoculum potential of soils from grazed sites.

Reductions in mycorrhizas in response to defoliation are expected as the typical result in more general treatments of this topic (Gehring & Whitham 1994, 2002; Wardle 2002; Wardle *et al*. 2004), simply because the underlying mechanism of competing carbon sinks is so appealing. However, this mechanism is evidently not always in effect, at least when percentage colonization of roots is used to quantify mycorrhizal benefit. Furthermore, the sporadically reported results of effects on other measures of fungal abundance, such as spores, sporocarps, extraradical hyphae and ergosterol levels, are also highly variable (Daft & El-Giehmi 1978; Allsopp 1998; Kuikka *et al*. 2003; Markkola *et al*. 2004). However, patterns are generally consistent with the results of our meta-analysis, with all reported cases where AMF spores, arbuscules, vesicles or extraradical hyphae were not affected or enhanced by defoliation occurring when host plants were perennial grasses or mixes of perennial grasses and forbs (Allen, Richards & Busso 1989; Bentivenga & Hetrick 1992; Allsopp 1998; Eom *et al*. 1999; Titus & Leps 2000; Dhillion & Gardsjord 2004; Klironomos, McCune & Moutoglis 2004).

An alternative mechanism whereby heavily colonized plants would be favoured after defoliation relates to the increased carbon exudation common following defoliation (Hamilton & Frank 2001; Hamilton *et al*. 2008). This increased flux of labile carbon to the rhizosphere stimulates microbial growth and nitrogen mineralization, increasing the available pool of nitrogen in the soil (Hamilton & Frank 2001; Ayres *et al*. 2004; Hamilton *et al*. 2008). Plants showing strong compensatory growth responses after defoliation, both grasses and trees, have higher leaf nitrogen content than non-defoliated plants (Hamilton & Frank 2001; Ayres *et al*. 2004; Hamilton *et al*. 2008). Leaf endophytes also confer greater benefit on their host plants in high-nutrient soils (Saikkonen *et al*. 2006), and mycorrhizal fungi could be responsible for scavenging the soil for these excess nutrients. This would select for the maintenance of

colonization even under heavy defoliation regimes so that plants could access the short-term pulse of mineralized nitrogen following foliage removal. This mechanism may also explain why clipping can effectively mimic grazing pressure in many cases. Much of the nitrogen absorbed by plants during compensatory regrowth is available due to physiological responses of the plant, and not due to urine and dung inputs from the grazing animals (Mikola *et al*. 2009).

In conclusion, our results challenge the carbon-limitation hypothesis and suggest that herbivory or clipping does not reduce mycorrhizal colonization by biologically meaningful levels in many types of plants, including commonly studied perennial grasses and forbs. We did not find strong negative effects in any plants, but we found strong positive effects of herbivory in mixtures of perennial grasses and forbs. However, only five experiments from three publications used mixtures of plants, so additional work must be carried out before concluding that this trend persists. Host plant type and treatment appear to influence the mycorrhizal response to herbivory, but further research is needed to verify this assertion since the moderators used in this analysis were confounded. Other moderators commonly confounded with plant type included type of mycorrhiza, fertilization and setting, but none of these moderators affected the effect size, further pointing to the importance of host plant type and herbivory treatment. Future experiments designed to compare specifically effects of different types of herbivory across plant types while holding other moderators constant would help to resolve these issues.

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