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The interactive effects of plant microbial symbionts: a review and meta-analysis

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Abstract In nature, plants often associate with multiple symbionts concurrently, yet the effects of tripartite symbioses are not well understood. We expected synergistic growth responses from plants associating with functionally distinct symbionts. In contrast, symbionts providing similar benefits to a host may reduce host plant growth. We reviewed studies investigating the effect of multiple interactions on host plant performance. Additionally, we conducted a meta-analysis on the studies that performed controlled manipulations of the presence of two microbial symbionts. Using response ratios, we investigated the effects on plants of pairs of symbionts (mycorrhizal fungi, fungal endophytes, and nitrogen-fixers). The results did not support the view that arbuscular mycorrhizal (AM) fungi and rhizobia should interact synergistically. In contrast, we found the joint effects of fungal endophytes and arbuscular mycorrhizal fungi to be greater than expected given their independent effects. This increase in plant performance only held for antagonistic endophytes, whose negative effects were alleviated when in association with AM fungi, while the impact of beneficial endophytes was not altered by infection with AM fungi. Generalizations from the meta-analysis were limited by the substantial variation within types of interactions and the data available, highlighting the need for more research on a range of plant systems.

Keywords Plant microbial symbiosis · Plant community dynamics · Species interactions · Arbuscular mycorrhizal fungi · Fungal endophytes · Nitrogen-fixers · Ecological meta-analyses

1 Introduction

The associations between plant hosts and their microbial symbionts are known to influence individual plant fitness (Schardl et al. 2004) and plant population dynamics (Bever et al. 1997). Consequently, these interactions play large and varied roles in the establishment and maintenance of plant community diversity and ecosystem properties (van der Heijden et al. 1998). There is a growing appreciation that the environmental context in which such interactions take place determine variation within interspecific interactions (van der Heijden et al. 2003; Thompson and Fernandez 2006; Vogelsang et al. 2006; Johnson 2010). Many studies focusing on variation in the abiotic environment demonstrate the context-dependent nature of symbioses, such as in the interaction between mycorrhizal fungi and plants (Reynolds et al. 2006; Vogelsang et al. 2006). However, the potential of the biotic environment (i.e. additional species within a community) to have similar impacts is often overlooked. In nature, plants often interact with multiple partners concurrently and the interactions between these symbionts can influence the dynamics of both host and symbiont populations.

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The presence of multiple microbial plant symbionts is likely to influence the nature of individual plant-symbiont interactions by altering the balance of trade (cost: benefit) relationship between the host and symbiont through competition for, or enhancement of, a common resource (Bronstein 1994). When multiple symbionts simultaneously interact with a common host, positive interactive effects may result if there are disproportional increases in benefits relative to the costs of both associations. This phenomenon is more likely to occur with functionally distinct symbionts that provide different benefits to the plant (Stanton 2003). For this reason, synergism is often expected, and sometimes found, between nutritionally complementary symbionts. Examples of synergistic interactions have been found with rhizobia and AM fungi that increase nitrogen and phosphorus availability, respectively (Jia et al. 2004). Likewise, if symbionts directly or indirectly interact to enhance each other's effectiveness, their co-occurrence can result in synergistic plant growth (Miller and Travis 1996). Associations between plants and some microbial symbionts may increase the host's ability to provide resources to additional symbionts though increased plant growth or resource quality (Bennett et al. 2006).

However, multiple interactions on a common host plant may lead to a decrease of the independent effects of each symbiont through depletion of host resources. Ultimately, reduction of host resources causes a decrease in host fitness that is greater than expected based on individual interactions. Antagonism between plant microbial symbionts might be expected as they compete for plant photosynthate (Harris et al. 1985). An antagonistic interaction maybe more likely if interactions are functionally equivalent, requiring the host to pay the cost of participating in each while deriving the benefits of only one association (Stanton 2003). Additionally, the presence of simultaneous associations has the potential to directly or indirectly interfere with an interaction (Miller and Travis 1996). For example, Mack and Rudgers (2008) demonstrated that fungal endophytes reduced the degree to which AM fungi infect plants. Conversely, another study showed that fungal endophyte infection enhances AM fungal colonization (Novas et al. 2005).

Previously, a study by Morris et al. (2007) investigated how the independent effect sizes of a wide range of plant mutualists and enemies are influenced by the presence of another interaction. Here, we specifically review the current understanding of the interactions between common microbial groups that live in symbiotic association with plants throughout their lifetimes: fungal endophytes, mycorrhizal fungi, and nitrogen-fixing microbes. In addition to reviewing past research, we conducted a meta-analysis on a subset of reviewed studies. Appropriate studies for inclusion into the meta-analysis evaluated the biotic environment of a plant by manipulating the presence of at least two of the three examined types of microbial plant symbionts, and recorded measures of plant growth including error within treatments. We analyzed overall trends in additive and non-additive effects on plants associating with two symbionts simultaneously.

2 Materials and methods

2.1 Data collection

We reviewed published studies that manipulated at least two microbial symbionts of plants in a fully-factorial manner and measured plant responses (e.g. biomass production, growth rate, leaf area). We found articles using Web of Science search terms: mycorrhiza* and "fungal endophyt*", mycorrhiza* and rhizob* and interact*, mycorrhizal* and nitrogen-fix* and interact*, and mycorrhizal* and *Frankia*, as well as searching cited references from review articles, including Morris et al. (2007). More recent articles were found by omitting the term "interact" from Web of Science searches and limiting articles to the year 2008. We included articles published through May 2008. From this search, 31 studies were found to be appropriate for inclusion in the meta-analysis (Supporting Information 1). Depending on the nature of the particular experimental design, many of these studies yielded multiple data points. For example, if a study included different experimental treatments (i.e. shade, moisture, or nutrients) we extracted multiple data points.

From each study we recorded data on the plant species examined, experimental design, type of symbionts manipulated, values of control treatments, independent effects of each symbiont on host performance, their interactive effects and measures of variance within treatments. For data presented in graphical format, we used ImageJ (rsbweb.nih.gov/ij/) to accurately measure means and errors. From the studies utilized, plant symbionts could be categorized as one of the following: AM fungi, ectomycorrhizal (EM) fungi, fungal endophytes, rhizobia (which includes members of the bacterial genera *Rhizobium* and *Bradyrhizobium*), and non-rhizobial nitrogen-fixers (e.g. *Frankia*) (Table 1). In this study, the fungal endophyte category consists of only the systemic foliar fungal endophyte species of grasses (Class 1 endophytes (Rodriguez et al. 2009)) and excludes non-systemic fungal endophytes of other herbaceous and woody species. In addition to evaluating these types of interactions, we also combined the effects of AM with EM fungi and rhizobia with other nitrogen-fixers to obtain measures of the effects of all mycorrhizal and all nitrogen-fixing symbionts, respectively. The availability of data constrained the meta-

Table 1 Summary of data included in this meta-analysis. Due to small sample sizes, some studies included in individual symbiont groups are not represented in symbiont interaction groups. Additionally, some studies incorporated into the combination of all mycorrhizae with all nitrogen fixers category are not included as individual interaction groups (i.e. EM fungi with nitrogen fixers)

Symbiont	Number of data points	Interactions	Number of data points
AM fungi	67	AM fungi-fungal endophyte	22
EM fungi	4	AM fungi — rhizobia	30
Fungal endophyte	22	AM fungi — N-fixer	15
Rhizobia	30	All mycorrhizae — All N-fixers	49
N-fixer	23		
All mycorrhizae	71		
All N-fixers	53		

analysis to three different pairs of plant symbionts: AM fungi with fungal endophytes, AM fungi with rhizobia, AM fungi with N-fixers (Table 1).

2.2 Data analysis

We calculated the independent, overall, and interactive effects for each type of symbiosis and symbiont pair (Morris et al. 2007). The independent effect of a single symbiont was calculated as the difference between the treatment with the symbiont present and the control treatment. As a complementary measure, we determined overall effect sizes by adding the plant responses of the two treatments in which the symbiont was present (i.e. in isolation as well as in combination with a second symbiont) and then subtracting the sum of the responses of plants in the treatments in which the symbiont was absent (i.e. control and the second symbiont in isolation). The interactive effect of a symbiont pair was calculated as the difference between the treatments with those symbionts present minus the effects of each symbiont independently. Independent and interactive effect sizes are calculated within individual studies with regard to the control. As such, studies utilizing different metrics of plant performance (e.g. biomass and leaf area) were combined into a common effect size.

To compare symbiont effects on plants across studies, we calculated the size of these effects in each study using Hedge's d and response ratios. Many meta-analyses utilize Hedge's d as it accounts for the variation associated with each data point by dividing the effect size by the pooled standard deviation of treatments (Hedges and Olkin 1985). Hedge's d effect sizes are reported in Supporting Information 2. We present response ratios, as these values provide a more intuitive measure of symbiont and interactive effects. The response ratio indicated the percentage of plant response increase or decrease that can be attributed to that treatment. For example, a response ratio of 1.1 signified a 10% increase in plant response when associated with the symbiont or pair. In statistical analyses, we used log-transformed response ratios as these are amenable to meta-analysis (Hedges et al. 1999). Response ratios for independent and overall effects were calculated as the log of the appropriate treatments in which the symbiont is present divided by the corresponding treatments in which the symbiont is absent. We defined interactive response ratios as the difference between the log of the treatment with the symbiont pair present divided by the effect of one symbiont and the log of the second symbiont's independent effect. Hedge's d effect sizes and response ratios were calculated using MATLAB codes as in Morris et al. (2007).

Once effect sizes were calculated for each group (the independent, overall, and interactive effects of each type of symbiosis and each combination of symbiosis), we tested for homogeneity of these effects sizes within a group using the Q statistic (Hedges and Olkin 1985). This test determines if effect sizes between different studies are similar enough to confidently combine them into a common effect size. In all cases, the calculated Q statistic suggested homogeneity in effect sizes. Studies were integrated by weighting the mean effect size by the inverse of the individual study variance (Gurevitch et al. 2001), thereby decreasing the influence of studies with relatively large variances on the group mean. We used *t*-tests to determine if weighted independent, overall, and interactive log response ratios were statistically greater than (indicating positive or greater than expected plant responses) or less than (signifying negative or less than expected plant responses) zero. Because the Q statistic is an incomplete test of the appropriateness of a group designation (Hedges et al. 1999), we also analyzed the unweighted means when we suspected that effect size variation within a group maybe explained by unaccounted for dissimilarities within the category. As fungal endophytes of grasses were found to be a particularly diverse category, we tested for differences in the distribution of unweighted response ratios

between endophytes that were beneficial and antagonistic with a Wilcoxon-Mann–Whitney rank sum test. The beneficial or antagonistic nature of the fungal endophyte was identified by comparing the effect of the endophyte in isolation on plant growth to the growth of plants in the sterile control treatment. This measure did not take into account the endophyte interactive effects with another symbiont. It was possible for this distinction to change within a study (e.g. if the study included a controlled manipulation of abiotic conditions).

3 Review of published articles

Our literature search revealed many studies that were relevant to the topic of interactive effects of multiple symbionts (Supporting Information 1). However, several of these studies did not meet the standards for inclusion into the meta-analysis. For example, some studies quantified aspects of the interactions that may relate to plant fitness (mycorrhizal colonization), but did not include more direct plant fitness measurements (i.e. biomass). Alternatively, some studies did not report appropriate measures of error or have all necessary controls to accurately compare plant responses to treatments. We present a summary of studies included into the meta-analysis as well as studies that we were unable to include (Table 2) and when possible we identify potential mechanisms for the interactive effects.

3.1 Fungal endophytes and AM fungal interactions

We found nine studies investigating the interactive effect of foliar fungal endophytes of grasses and AM fungi. Four of these investigations focused on the direct interaction between the symbionts in relation to mycorrhizal colonization rates or fungal endophyte hyphal density. Three studies reported a negative interaction between fungal endophyte infection and AM fungal colonization. In contrast, one study found fungal endophyte-infected plants to have higher rates of AM fungal colonization than endophyte-free plants (Novas et al. 2005). The possible mechanisms for this interaction are undetermined. However, studies showing interference between the symbionts speculate that the alkaloid production of fungal endophytes has allelopathic effects on AM fungi.

Studies that measured plant fitness demonstrated a variety of plant responses to simultaneous infection by AM fungi and fungal endophytes. Two studies reported negative plant responses with both symbionts. Muller (2003) found mixed results with different plant cultivars and strains of fungal endophyte. Two additional studies found no clear effect of the co-occurrence of fungal endophytes and AM fungi on a common host. Several of these studies reported measures of symbiont response in addition to plant fitness. For example, two studies found that fungal endophyte infection reduced AM fungal colonization (Muller 2003; Omacini et al. 2006), and two reported decreased resistance to herbivory from endophyte-infected plants when in association with AM fungi (Barker 1987; Vicari et al. 2002). While these studies identified possible mechanisms for interactive effects related to host fitness (e.g. reduced AM fungi colonization and herbivore resistance), there was no clear direct effect on plant fitness from either measure.

Table 2 Summary of distribution of reviewed articles indicating the number of studies having mainly positive, negative, mixed, or no interactive effects within symbiont combinations. Studies having two main results (i.e. within the same study, some plant symbiont combinations result in a positive interaction and others result in a negative interaction) are represented in more than one effect type. In the fungal endophyte and AM fungi combination, studies are divided between those that directly measured plant response and those that measured symbiont response (i.e. AM fungi colonization rate)

	Positive	Negative	Mixed	None
Fungal endophytes and AM fungi (plant responses)	0	2	1	2
Fungal endophytes and AM fungi (symbiont response)	1	3	0	0
AM fungi and Rhizobia	32	3	11	5
All mycorrhizae and non-rhizobia nitrogen fixers	8	5	3	2

3.2 Mycorrhizal and nitrogen-fixing symbionts

Thirty-two of the 51 studies examining AM fungi and rhizobia interactions reported that plants infected with both symbionts had greater plant responses than either symbiont independently or sterile control plants. In addition to plant fitness responses, many of these studies also reported an increase in nodule activity and nutrient uptake when plants were associating with both AM fungi and rhizobia, as well as an increase in AM fungal colonization. However, two studies found that AM fungal colonization decreased in the presence of rhizobia, but Jia et al. (2004) also demonstrated that the photosynthetic rate was higher in plants infected with both symbionts concurrently. Similarly, Niranjan et al. (2007) found that while AM fungal colonization decreased with rhizobia, nitrogen fixation was higher in the presence of both symbionts. While many of the interactions were positive, they were not usually synergistic (but see Pacovsky et al. 1986; Ferrari and Wall 2008), as plants often did not perform better than expected given symbiont individual effects.

In several studies, including five showing some positive responses, interactive effects depended upon the specific

combination of species and/or strains of AM fungi and rhizobia involved in the interaction. While it remains unexplained why certain symbiont combinations result in a variety of plant responses, it appears that the interaction between AM fungi and rhizobia maybe highly dependent upon the nutrient level of the soil (Ames and Bethlenfalvay 1987; Pan and Cheng 1988; Ianson and Linderman 1993). AM fungi are capable of nitrogen as well as phosphorus uptake, and may also be able to stimulate nitrogen fixation through increased provisioning of phosphorus. Only three studies demonstrated negative interactive effects of AM fungi and rhizobia. One (Bethlenfalvay et al. 1982), attributed the inhibitory effect of AM fungi on rhizobia nitrogen-fixation efficiency and plant growth reduction to competition between the symbionts for phosphorus and plant photosynthate.

Five of the 18 studies involving the interactions between AM or EM fungi and non-rhizobial nitrogen-fixing symbionts identified negative plant responses to dual inoculation. The negative interaction may be attributed to a general lack, or negative effect, of mycorrhizal fungal inoculation on plant responses in some studies. In one experiment, non-mycorrhizal plants had higher biomass than mycorrhizal plants, but both groups benefited from higher phosphorus availability (Ekblad et al. 1995). Some evidence also points to an inhibitory effect of *Frankia* on mycorrhizal colonization (Vonderwell and Enebak 2000).

In contrast, eight studies reported positive interactions between mycorrhizal fungi and non-rhizobia nitrogen-fixing symbionts. Several of these studies reported increased efficiency in nutrient uptake (e.g. nitrogen fixation or mycorrhizal colonization) from one or both symbionts when the host was infected with both. Fragabeddiar and Letacon (1990) found a positive interaction between *Frankia* and AM fungi that may be mediated through AM fungi's ability to stimulate nitrogen fixation. Nitrogen fixation was also enhanced by phosphorus fertilization, but not as strongly as the addition of AM fungi, suggesting that AM fungal associations offer more than phosphorus provisioning to increase nitrogen fixation. Jha et al. (1993) also found that results depended upon soil phosphorus levels, with the strongest positive interactive effects occurring at both the highest and lowest phosphorus levels.

4 Results: meta-analysis

Our meta-analysis included three broad types of interactions between three different combinations of microbial symbionts (Table 1). We found no indication of publication bias after plotting response ratios of independent and interactive effect sizes verses sample size for each study. Fungal endophytes were the only symbiont group included in this study that decreased plant performance independent of other interactions (t_{21}=-2.42; P=0.03). In contrast, nitrogen-fixing symbionts significantly increased plant performance. Inoculation with either rhizobia or other types of nitrogen fixers significantly increased plant response compared to uninoculated controls (t_{29}=2.54; P=0.01 and t_{22}=3.98; P=0.001, respectively). Across studies involving AM fungi, plant responses were generally positively affected by inoculation but not statistically different from zero (Fig. 1). Generally, independent effects were not altered when testing across biotic environments (Supporting Information 3). However, the overall effect sizes of both AM fungi and all mycorrhizal interactions combined are significantly beneficial to plant responses (t_{66}=4.12; P< 0.001; t_{70}=4.05; P<0.001). The overall effect size of the fungal endophyte group is no longer significantly antagonistic when combining across biotic environments. Although our meta-analysis identifies generalities between types of symbionts, individual value plots for each symbiont show a wide range of variation in the response ratios within significant and non-significant effects (Fig. 2).

The interactive effects of fungal endophytes and AM fungi resulted in positive plant responses that were greater than expected given independent responses (t_{21}=3.38, P= 0.003). While the average weighted response ratio for plants simultaneously infected with AM fungi and rhizobia, as well as AM fungi and other nitrogen-fixers, was negative, these interactive effects did not differ in their response from additive expectations (Fig. 3). Individual

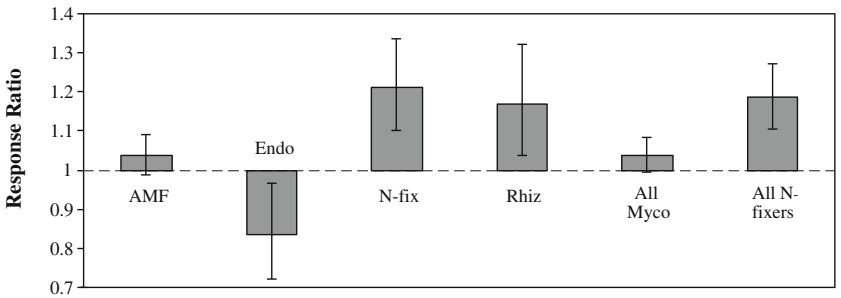

Fig. 1 Weighted independent response ratios of symbiont groups with 95% confidence intervals. The 1 line indicates no plant response when interacting with a symbiont. Positive and negative responses fall above and below the 1 line, respectively

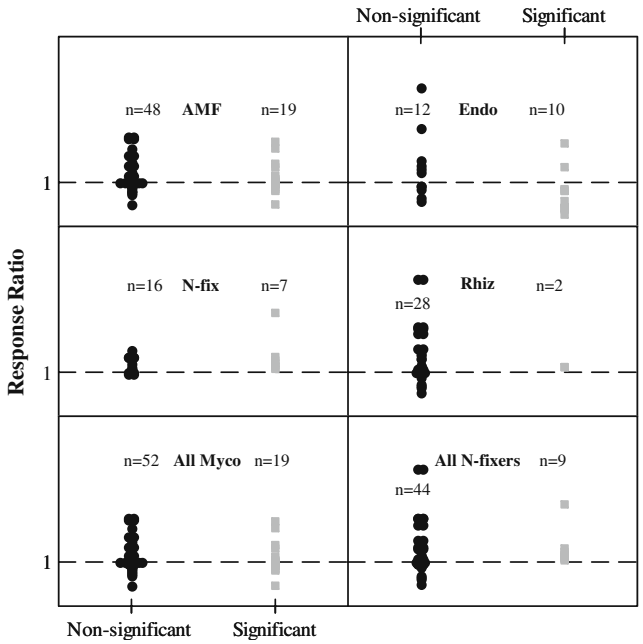

Fig. 2 Individual value plots for each symbiont group with sample sizes. Symbiont groups are divided between data points with significant interactive effects in either direction (*grey squares*) and non-significant interactions (*black circles*)

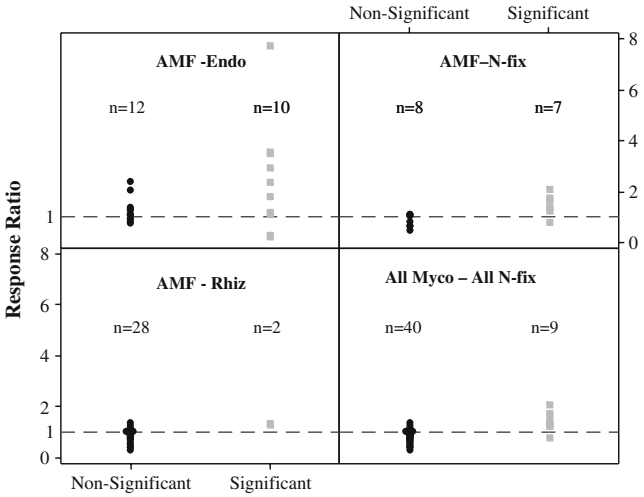

Fig. 4 Individual value plots for interactions with sample sizes. Symbiont interaction groups are divided between data points with significant interactive effects in either direction (*grey squares*) and non-significant interactions (*black circles*)

value plots demonstrate that, as with the independent effect sizes, most of the data (~75%) utilized to determine interactive effects were non-significant (Fig. 4). While many of the interactive effects of several symbiont combinations (e.g. AM fungi with nitrogen-fixers and with fungal endophytes) decreased plant performance, studies with responses greater than expected tended to have significant effect sizes (Fig. 4). Weighted response ratios showed no difference from additive interactions between mycorrhizal and nitrogen-fixer interactions, while unweighted interactive response ratios of mycorrhizal with nitrogen-fixers were marginally less than expected given the symbionts' independently beneficial effects (t_{48}= -1.82; P=0.075).

As species of fungal endophytes of grasses vary between beneficial and antagonistic interactions with their host plants (Ahlholm et al. 2002; Cheplick 2007), we tested whether the direction of the interactive effect varied with the nature of the main endophyte effect. Separating interactive response ratios of fungal endophytes and AM fungi based on comparing the effect of fungal endophyte infection alone versus the sterile control, revealed differences in interactive effects between relatively mutualistic versus more parasitic endophyte groups (t_{16}=4.17; P= 0.001). When the symbiont combination included antagonistic fungal endophytes, the interactive effect was greater than predicted from independent effects (t_{12}=4.57; P= 0.001). Plants inoculated with AM fungi and paired with more mutualistic endophyte associations tended to have reduced benefits, but these response ratios were not significantly different from the expected additive responses (Fig. 5).

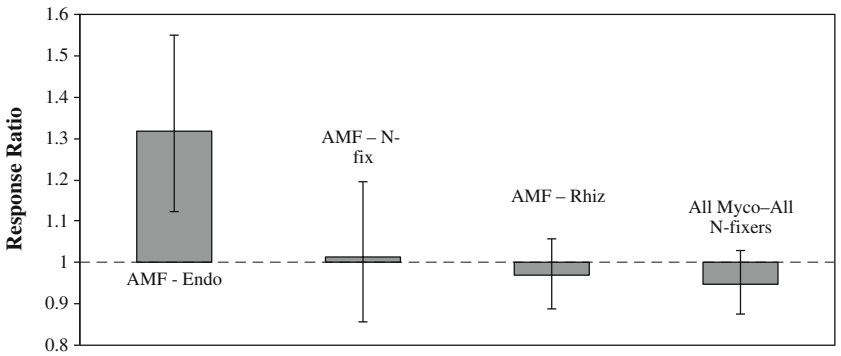

Fig. 3 Weighted interactive response ratios with 95% confidence intervals. Intervals crossing the 1 line represent cases where the combination of symbionts results in additive interaction effects. Intervals above or below the 1 line indicate synergistic or antagonistic interactive effects of simultaneous symbionts, respectively

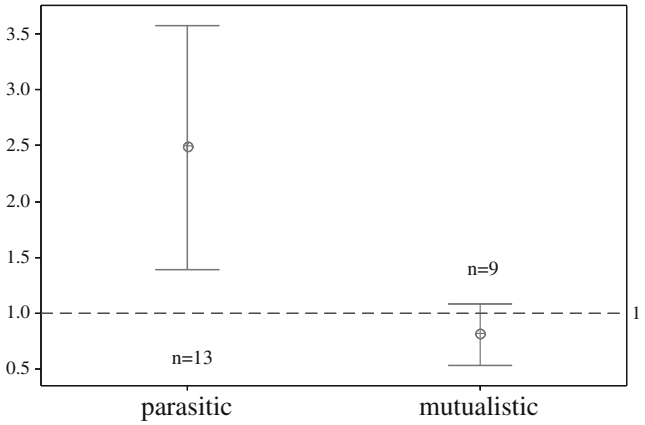

Fig. 5 Unweighted interactive response ratios for AMF and fungal endophyte interactions with 95% confidence intervals. Responses are grouped between interactions involving independently mutualistic and parasitic fungal endophyte associations

5 Discussion

We found that the majority (5/9) of studies investigating the interactions between AM fungi and fungal endophytes demonstrated a negative interaction between the two symbionts. While interactions between mycorrhizal fungi and nitrogen-fixing symbionts were generally positive, we did not find evidence for synergistic responses in plant growth. In general, interactive plant response ratios were not different from the additive expectations predicted from the meta-analysis. In the case of mycorrhizal and nitrogen-fixing associations, the lack of an interactive effect is especially surprising, as the co-occurrence of symbionts providing non-equivalent essential resources, such as phosphorus and nitrogen, are expected to demonstrate synergistic interactive effects (Barea et al. 2005). Rather, while mycorrhizal and nitrogen-fixing symbionts were often independently beneficial, plants associating with each of these symbionts tended to underperform when analyzing across all studies. This result was supported in the review, where plants often had the highest responses when inoculated with both mycorrhizal and nitrogen-fixing symbionts, but not meeting the expected combined effect of each symbiont independently.

Synergism between AM fungi and nitrogen fixers would only be expected in environments in which both nitrogen and phosphorus were limiting. Our observation of the absence of synergistic interactive effects may reflect the nutrient conditions of the experimental soil. Microbal partners are expected to be more beneficial to plants in areas of poor environmental quality (Johnson et al. 1997). If experimental plants did not experience nutrient limitation, both AM fungi and nitrogen-fixers could provide a marginal benefit independently. When plants interacted with both types of symbionts, the marginal benefits may have been weakened due to the higher demand and competition for plant photosynthate. Additionally, many studies on AM fungi — nitrogen-fixer interactions were conducted on agriculturally important plants (i.e. soybean). Under high nutrient conditions, such as highly fertilized areas, plants have been shown to adapt reduced dependency on mycorrhizal colonization relative to plants from low-nutrient environments (Schultz et al. 2001). Simillarly, symbionts experiencing high-input environments can undergo selection for less beneficial strains that are adapted to a decreased host investment (Johnson 1993). By focusing on both plants and microbes that have evolved in high input environments, studies may be using plant and microbial genotypes that limit investment in the interaction, therefore decreasing the likelihood of synergistic interactive effects.

We were unable to test for dependence of interactions on abiotic environmental and experimental conditions because of insufficient data. Tests of environmental dependence would provide clues as to the mechanism for non-additive effects. Our inability to test for environmental dependence illustrates the need for more experimental studies on the effects of multiple symbionts on plant performance, as well as careful recording of environmental conditions associated with these studies. An alternative explanation for the absence of synergisms is that these symbioses, while appearing to specialize in providing non-equivalent resources, may actually be functionally similar, as arbuscular mycorrhizal fungi have been shown to provide plants with nitrogen, as well as phosphorus, in some cases (Govindarajulu et al. 2005).

Additionally, in some studies maximum productivity of plants could have been limited by water, light availability, or micronutrient levels. It is also important to consider the growth stage of plants under investigation. Often, mycorrhizal associations that are shown to be costly to plants at early stages of growth provide long-term benefits to plants as they mature and are more able to support the symbiosis (Johnson et al. 1997). Given the time and space constraints of long-term studies, it is reasonable that the full benefits of mutualistic associations are not attained within the time frame of many ecological experiments. Variation in the effects of a symbiont throughout a growing season and a plant's lifetime has strong implications for the interactive effects of additional symbionts on plant responses. For example, Orfanoudakis et al. (2004) speculates that the inhibitory effects of *Frankia* and AM fungi on early growth may result from competition between the symbiont for plant resources, but that as the plant matures and gains higher photosynthetic ability the detrimental effects could be reduced.

Many of the studies presented in this review were conducted on economically important plants or biological model systems under artificial conditions that omit the natural context and coevolutionary history between the

hosts and symbionts. We found evidence that the strength and nature of interactive effects varies for different plant and microbial genotype combinations, even over small spatial scales. Turkington and Harper (1979) found that clover grew better when in competition with a natural neighbor than with other plants taken from the same grassland, regardless of the competitor's identity. Later work in this grassland identified that the interaction between neighboring plants is partially mediated by rhizobia bacteria. The rhizobia harbored by neighboring plants better promote the growth of clover genotypes than does the rhizosphere bacteria of non-neighboring plants (Chanway et al. 1989). While this experiment isolates the effect of the clover-rhizobia interaction, experimental plants were not sterilized and may have contained other naturally-occurring soil microbes. If mycorrhizal associations were unintentionally included, it is possible that the growth enhancement between neighboring plants may contribute to rhizobia-mycorrhizal synergistic interactions.

Plants infected with both AM fungi and fungal endophytes generally responded better than expected (Fig. 2). Class 1 fungal endophytes of grasses make up a group of symbionts known to be highly variable in their effects on host responses (Rodriguez et al. 2009). Taking into account whether endophyte infection resulted in a growth enhancement or depression revealed that the dynamics between a host and single symbiont can have large impacts on the host response to simultaneous interactions. When paired with fungal endophytes that decreased host fitness when inoculated singly, the interaction between the endophyte and an AM fungal association was more positive than when the combination included an independently beneficial fungal endophyte (Fig. 5). Although AM fungi and fungal endophytes are functionally distinct and spatially separate within a common host plant, they may interact in currently unidentified, yet meaningful, ways to influence host plant responses. As demonstrated from reviewing additional publications, a few studies have investigated direct effects of fungal endophyte infection on mycorrhizal colonization, producing mixed results. The substantial variation that we observed in endophyte behavior is particularly notable given the limited taxonomic diversity of fungal symbionts examined thus far.

A few well-studied, agronomically important systems, such as the *Neotyphodium-Lolium* interaction, are heavily represented in the database. More work on the interactive effects of a wider diversity of fungal endophytes with AM fungi is required to confirm the pattern of synergism observed in published papers. Additionally, we found that plants infected with fungal endophytes experienced a reduction in growth relative to uninfected plants independent of additional symbionts. This result is dissimilar to the majority of studies of grass-endophyte symbioses, which often demonstrate an increase in fitness of endophyte-infected compared to uninfected plants (Clay 1988). It is important to note that the data contributing to this result was taken from a minority of grass-endophyte studies that manipulated a second symbiont along with fungal endophyte infection. The lack of sufficient data to test for the role of abiotic environmental conditions could also explain the overall growth depression of plant infected with fungal endophytes verses endophyte-free individuals. Many benefits of grass-endophyte associations are not apparent under stress-free environments, which is likely to be the case in greenhouse studies that attempt to reduce the effects of stress on plant growth.

Our study illustrates one challenge in the application of meta-analyses in ecology. Fungal endophytes proved to be a heterogeneous category, in which beneficial and non-beneficial endophytes interact in qualitatively different ways with mycorrhizal fungi. Lumping endophytes into a single category, one would have concluded that endophytes are generally significantly antagonistic to plant growth and these antagonistic effects are enhanced by association with AM fungi. Dividing the endophytes into independently beneficial and non-beneficial categories we found that, in contrast to antagonistic endophytes, the effect of beneficial endophytes tends to be dampened by simultaneous association with AM fungi. An appreciation for the type and nature of data being evaluated can allow researchers insight into informative groupings regardless of statistical confidence that studies represent a single category.

Thus far, studies of interspecific interactions focused primarily on the dynamics occurring between a host and a single type of symbiont. Previous research has demonstrated that diverse microbial symbionts form associations with plants, and that the nature of these associations varies depending on host-symbiont species identity and genotype (Klironomos 2003), nutrient resource levels (Johnson et al. 1997), and other abiotic environmental conditions (Tintjer et al. 2008). In nature it is common for both hosts and symbionts to engage in multiple symbiotic interactions concurrently. More recent research has shifted focus to include aspects of both the abiotic and biotic environment as factors influencing population and community-level dynamics (Vogelsang et al. 2006). The results of the meta-analysis are constrained by limitations in available data, and thus may not reflect the dominant patterns in nature. However, these results do reveal that commonly held expectations for synergies between microbial symbionts were not generally supported by the existing literature.

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