Symbiosis and the Regulation of Communities

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SYNOPSIS. Ecologists have long been interested in factors that control the structure of communities and the relative importance of top-down effects of predators versus bottom-up effects of resources. There is a growing body of evidence that microbial symbioses are important determinants of plant community structure and indirectly affect herbivore and predator trophic levels. Studies with mycorrhizal fungi, N-fixing bacteria and endophytes of grasses have demonstrated that they can affect competition, coexistence, soil nutrient dynamics and plant-herbivore interactions. Long-term field experiments with one grass/endophyte interaction suggest that grassland community structure is determined by the fungus. While total plant productivity of experimental plots was similar, the composition of the vegetation was altered by endophyte symbiosis. The host grass tall fescue (Festuca arundinacea) dominated plots when infected while other grasses greatly increased in uninfected plots. Indirect evidence suggests that changes in prairie vole (Microtus ochrogaster) grazing patterns and reproductive physiology may be in part responsible for vegetational changes. These results provide evidence that, in addition to bottom-up and top-down forces, microbial symbionts of plants are important determinants of community structure.

INTRODUCTION

The factors that control the structure of communities have been of longstanding interest to ecologists. Much attention has been given to the relative importance of top-down effects of predators versus bottom-up effects of resources on community structure. We now recognize that both types of forces are probably acting simultaneously in most communities, raising the question of what factors determine the relative importance of each type of effect (Hunter and Price, 1992; Strong, 1992). Largely ignored in this discussion is the potential for symbiotic microorganisms to regulate terrestrial communities.

Historically, symbiosis has been ignored from both theoretical and empirical perspectives relative to interactions like competition and predation. Given the difficulty in quantifying and manipulating symbiotic interactions in the field compared to other types of species interactions, it is not surprising that symbiosis is rarely considered in discussion of community regulation. Nevertheless, recent studies have clearly demonstrated that microbial symbionts of plants can affect the structure and function of plant communities (van der Heijden et al., 1998; Clay and Holah, 1999).

The objectives of this paper are two-fold. First, I briefly develop the hypothesis that heterotrophic microbes existing symbiotically within plants can regulate primary producer and primary consumer trophic levels, and present evidence for this hypothesis from published studies. Second, I review recent results from ongoing research comparing the diversity and productivity of experimental tall fescue grasslands that are subject to vole herbivory and differ in endophyte infection.

Top-down and bottom-up effects

The Fretwell model (Fretwell, 1977, 1987; Oksanen et al., 1981), illustrates both top-down and bottom-up forces (Fig. 1). As resources and primary productivity increase, the number of trophic levels that can be supported also increases. The number of trophic levels in turn determines the nature of regulation of the basal trophic level. In
the absence of herbivores (one trophic level), the plant community appears green given that there is no consumption of plants. With two trophic levels the plant community is grazed to low levels because herbivore populations grow unchecked except by their food supply. With three trophic levels the plant community again appears green because predators limit herbivore populations, allowing the basal trophic level to increase to the extent that resources allow. This pattern represents the well-known Hairston, Smith and Slobodkin (HSS) (1960) model and may be the general case in many terrestrial communities (Schmitz et al., 2000; Halaj and Wise, 2001). Finally, with four trophic levels, secondary predators control primary predators, reducing predation on herbivores, which then consume plants to a greater extent, as in two trophic-level systems.

Competition for limiting resource levels implicitly limits the number of trophic levels in this model (bottom-up effects) and can clearly control standing plant biomass in a number of systems. For example, Rosenzweig (1968) found that net primary productivity of terrestrial habitats was strongly predicted by evapotranspiration. In Michigan old fields, plant productivity significantly increased following nitrogen fertilization (Foster and Gross, 1998). Similarly, in California river systems, primary productivity was inversely related to forest canopy cover, which intercepts sunlight (Feminella et al., 1989). Biomass of herbivores and predators may also reflect resource levels and plant biomass. In African savannas both net primary productivity and large herbivore biomass increase with annual rainfall (Coe et al., 1976). The relationship between resource levels and abundance of different trophic levels in aquatic systems was illustrated by Wootton and Power (1993). Experimental shading of portions of a stream reduced biomass of algae and predators while greater light levels increased biomass of both groups. Herbivore biomass was unaffected by light levels in agreement with the predictions of Oksanen et al. (1981) for a three trophic-level system.

The best examples of top-down effects, or trophic cascades (Paine, 1980), come from aquatic communities. Manipulations of fish in a California river by Power (1990) demonstrated their strong indirect effects on filamentous green algae. Experimental cages containing fish resulted in greatly reduced algal biomass as a result of heavy infestations by herbivorous chironomid larvae. In contrast, cages lacking fish developed significantly greater algal biomass and cover, with lower densities of chironomids (Power, 1990). When large fish were excluded, predatory insects and fish fry were freed from predation and more effectively controlled chironomid populations feeding on algae. The observed pattern supports the Fretwell and HSS models (see also Carpenter et al., 1985).

Trophic cascades have been observed or inferred in terrestrial systems as well. Spiller and Schoener (1990) examined a simple island food web consisting of lizards, spiders, herbivorous arthropods and plants and created enclosures either with or without lizards. Damage to plants by herbivores was reduced when lizards were present, even though lizards also fed on predatory spiders. However, in another study they found no effect of lizard removals on plants (Spiller and Schoener, 1994) because the increased abundance of web spiders had similar effects on herbivorous arthropods. In a temperate woodland, Marquis and Whelan
(1994) manipulated avian predation of herbivorous insects by netting white oak saplings to exclude birds. Caged saplings had higher insect densities, greater leaf area consumed and lower biomass than uncaged saplings. Thus, birds had strong top-down effects on white oak trees mediated through insect predation. Similarly, Dyer and Letourneau (1999) manipulated a secondary predator in a complex, four-level tropical system. The presence of predatory beetles increased leaf damage to host plants because beetles attacked symbiotic ants that normally guard host plants against herbivorous insects.

Several recent reviews and meta-analyses have investigated the prevalence of trophic cascades in terrestrial communities. Most published studies used in these analyses were on herbaceous plant communities and arthropod herbivores where predator densities were experimentally manipulated. Schmitz et al. (2000) found that trophic cascades occurred frequently in terrestrial systems (45 of 60 tests). However, top-down effects of predators often attenuated when plants exhibited anti-herbivore adaptations or when the diversity of herbivore species was high. Further, the indirect effects of predators on plants was stronger when measured as plant damage rather than plant biomass, suggesting that plants have compensatory mechanisms that maintain growth despite damage. In another meta-analysis, Halaj and Wise (2001) also found strong evidence for trophic cascades in arthropod-dominated food webs. In most studies the removal of predators resulted in an increase in herbivore density and plant damage. Similar to Schmitz et al. (2000), they found little evidence for changes in plant biomass although several clear cases from simple agricultural systems occurred. In a related meta-analysis of the interaction between competition and predation in terrestrial field experiments, Gurevitch et al. (2000) found that predation reduced the strength of competitive interactions relative to predator-free conditions and that there was a significant interaction between competition and predation overall. In this study, most cases were from aquatic communities, often amphibian communities.

**Microbial Symbionts of Plants**

It is clear that both top-down and bottom-up effects can influence the structure of natural communities. It has been suggested that microbial symbionts of plants can affect the relative strength and direction of top-down and bottom-up forces (Hunter and Price, 1992; Polis and Strong, 1996). Microbes that exist on living plants or their products occupy a trophic position analogous to herbivores. They can influence primary productivity through their ability to produce or capture limiting resources and alter herbivore feeding patterns and population dynamics (see Fig. 2). The regulation of plant community structure by symbiotic microorganisms deserves further attention.

Plants make up the largest component of biomass in terrestrial systems and most exist symbiotically with mycorrhizal fungi (Smith and Read, 1997). Sanders et al. (1995) estimated that 225,000 plant species in 11,000 genera form arbuscular mycorrhizas. While much variation exists in the physical nature of mycorrhizal symbioses, the specific identities of plant and fungus, and the exchange of resources between partners, the host plant’s ability to uptake phosphorus at low concentrations is generally enhanced by mycorrhizal symbiosis (Allen, 1991). In turn, mycorrhizal fungi obtain carbon compounds from their host plants.

Symbiotic N-fixing bacteria are also widespread in the plant kingdom, although their distribution is not as cosmopolitan as that of mycorrhizal fungi (Akkermans and van Dijk, 1981). Nitrogen is generally the mineral nutrient most limiting to plant growth. Rhizobia occur in the root nodules of many legumes where they fix atmospheric nitrogen into a form that can be used by plants (Bergersen, 1982). In addition, N-fixing actinorhizal symbioses occur in a wide range of woody plant families (Smith and Douglas, 1987). More recently, symbiotic N-fixing bacteria have been discovered colonizing stems of several tropical grasses (James and Olivares, 1998). All of these bacterial symbionts also obtain carbon compounds from plants.

Many cool-season grasses are infected by
Fig. 2. Food web involving endophyte-infected grasses with top-down and bottom-up effects. Solid arrows represent direct effects and dashed arrows represent indirect effects. The endophyte has direct effects only on its host grass but indirect effects on competing plants, herbivores or predators.
endophytic fungi in the Ascomycete family Clavicipitaceae. The systemic fungi occur intercellularly in leaves, stems, and reproductive organs, but not in roots (Clay, 1988; Bacon and Hill, 1997). These endophytes are not known to spread contagiously but instead are disseminated through seeds and by vegetative growth of the host. In tall fescue (Festuca arundinacea), a well-studied species, endophyte-infected plants have increased drought tolerance, growth and seed production (see Clay, 1998). Like related Claviceps species, the endophytes produce alkaloids that can confer resistance to vertebrate and invertebrate herbivores (Bush et al., 1997). As with the other plant-microbial symbioses, endophytes obtain carbon compounds from host plants.

**Effects of plant-microbial symbiosis on primary productivity**

Given that plant growth is frequently limited by soil nitrogen or phosphorus, by making these nutrients more available to their hosts, mycorrhizal fungi and N-fixing bacteria can increase plant production. Most uptake of phosphorus from the soil by plants is actually through mycorrhizae, which also transport a variety of other mineral nutrients including K, N, S and Zn, as well as water (Allen, 1991). Mycorrhizal fungal hyphae have a very large surface-to-volume ratio, can efficiently permeate the soil (up to 50 m hyphae per mg soil) increasing the effective area of a plant’s root system, and can transport P several centimeters to meters (Woods and Brock, 1964; Rhodes and Gerdemann, 1975; Allen, 1991). By increasing host-plant resources, mycorrhizal fungi can increase photosynthetic rates (Trent et al., 1989), productivity (Last et al., 1990; Hartnett et al., 1993) and seed production (Stanley et al., 1993). Growth effects vary with stage of the life cycle, soil nutrient concentrations and mycorrhizal dependency of co-occurring species (Koide, 1991; Hartnett et al., 1993; Johnson et al., 1997; van der Heijden et al., 1998).

In a prairie community, suppression of mycorrhizal fungi with fungicides resulted in reduced dominance of C-4 grasses, increases in subordinate C-3 grasses and greater species diversity, but did not alter net productivity relative to control plots (Hartnett and Wilson, 1999). In microcosm communities inoculated with mycorrhizal fungi, forb species that are normally mycorrhizal in the field showed significantly higher survival and yields than in comparable nonmycorrhizal treatments (10 times higher in Plantago lanceolata) (Grime et al., 1987). Two species inhibited by mycorrhizal fungi were species that normally do not form mycorrhizal associations. van der Heijden et al. (1998) recently demonstrated in another microcosm experiment that certain species could not persist in mixed communities in the absence of mycorrhizae. Indeed, certain biomes (e.g., boreal forests) are dominated by plant species obligately dependent on mycorrhizal symbioses (Smith and Read, 1997). Thus, productivity of host species and their relative abundance in communities are affected by mycorrhizal symbiosis.

Considering N-fixing bacterial symbioses, growth of host plants is enhanced through increased supply of this limited nutrient and total community productivity may increase with increasing soil fertility. As with mycorrhizal associations, the absence of the appropriate bacterial symbiont in the soil can prevent dependent species from establishing or persisting in the community (Bottomley, 1992). In greenhouse pot studies, host plants grew significantly better when inoculated with rhizobia compared to uninoculated controls (Bottomley, 1992; Wilkinson and Parker, 1996). Similarly, in greenhouse competition studies, legume hosts grew better in competition with grasses when inoculated with rhizobia (de Wit et al., 1966; Turkeington et al., 1988). In many managed grasslands, sustained productivity depends on the maintenance of N-fixing clover species (Wilson, 1978). The phenomenon of overyielding is often found in mixtures of grasses and legumes (Hall, 1974; Harper, 1977), implying that additional mineral resources provided by symbiosis increases plant productivity and overall carrying capacity.

Increased levels of N in the soil resulting from symbiosis may allow certain species requiring high soil fertility to invade the...
site, thereby facilitating successional changes. For example, in Alaska alder trees (*Alnus*) and their symbiotic bacteria increase soil N levels on river sand banks and on soils newly exposed by retreating glaciers, leading to their replacement by conifer species with higher N requirements (Van Cleve *et al.*, 1971). The presence of native N-fixing lupines facilitates invasion by weed species in coastal California communities (Maron and Connors, 1996). Similarly, in Hawaii the alien actinorhizal plant *Myrica faya* increases soil N levels and allows the invasion of a number of additional non-native species (Vitousek *et al.*, 1987).

Endophyte symbiosis with grasses is also often associated with increases in host growth although the mechanism is not directly related to mineral nutrition, as with mycorrhizal fungi or N-fixing bacteria. For example, in a greenhouse experiment three sequential harvests of tall fescue and perennial ryegrass (*Lolium perenne*) were made over a 14-wk period to follow plant growth rate (Clay, 1987). Endophyte-infected ryegrass had significantly higher biomass than uninfected plants at each harvest. In tall fescue there was no difference the first harvest but an accelerating growth advantage for infected plants over time. In a review of published studies with tall fescue (Clay, 1997), infected plants outyielded uninfected plants in 14 of 15 comparisons. Only one study has demonstrated decreased growth of infected plants when seedlings were grown in nutrient-poor soil (Cheplick *et al.*, 1989). Growth of infected seedlings exceeded that of uninfected seedlings at moderate and high nutrient levels, and infected adults outperformed uninfected plants over all nutrient conditions. Several other studies have demonstrated growth advantages to endophyte-infected ryegrass (Latch *et al.*, 1985b; Clay, 1987; Cheplick *et al.*, 1989; Clay *et al.*, 1993), but other studies did not find any consistent effect of infection on growth (Keogh and Lawrence, 1987; Lewis and Clements, 1990). Variable results could reflect plant genotype × infection interactions, which have been found in several studies (Lewis and Clements, 1990; Marks and Clay, 1996; Cheplick, 1998).

Most research on effects of endophyte infection on growth have focused on tall fescue and perennial ryegrass. Other research suggests that endophyte-enhanced growth occurs more widely in grasses, although there is a clear need to examine a wider range of species and environmental conditions. In experiments with meadow fescue (*F. pratensis*), Malinowski *et al.* (1997a, b) found either no difference or greater growth of infected plants. In red fescue (*F. rubra*), endophyte infection enhanced plant survival in a common garden but did not have any effect on vegetative growth (Bazely *et al.*, 1997). However, Harberd (1961) found that infected clones of this species were far larger than uninfected clones, suggesting that they had greater growth and/or survival. In a greenhouse study, infected red fescue produced more biomass than uninfected plants in the presence of insect herbivory, but less biomass in the absence of herbivory (Clay *et al.*, 1993). Bier (1995) grew the native woodland grasses *F. obtusa* and *Poa sylvestris* under a range of conditions in the greenhouse and found that endophyte-infected plants were more productive than uninfected plants under most conditions. Given the large number of grass hosts and their distribution (Clay, 1997), grass-endophyte symbioses have the potential to affect plant productivity in a variety of habitats.

**Effects of plant-microbial symbiosis on herbivores**

While mycorrhizal and N-fixing bacterial symbioses are generally considered to be associated with nutrient uptake, studies have indicated that they can affect plant-herbivore interactions. Greater nutritional quality of host plants could cause higher levels of herbivory but, by improving plant growth and nutrition, these symbioses could also improve tolerance to herbivore damage. However, by reducing plant leaf area and photosynthetic capacity, herbivory may reduce the ability of host plants to support their symbionts (Gehring and Whitham, 1991; Johnson and Bentley, 1991). Alternatively, these symbioses may indirectly reduce herbivory by inducing changes in host-plant tissues such as concentrations of defense compounds.

Mycorrhizal symbiosis reduces herbivory...
of host plants in several systems. Infection of soybeans with the VAM fungus *Glomus fasciculatum* resulted in lower survival, reduced weight gain, and increased developmental time of two lepidopteran species compared to non-mycorrhizal soybeans (Rabin and Pacovsky, 1985). Gange and West (1994) found that foliar-feeding insects of *Plantago lanceolata* caused more damage when mycorrhizae were reduced by fungicide applications (see also Gange et al., 1994). Treated plants had lower concentrations of iridoid glycosides, known insect feeding deterrents. Several studies have also indicated that mycorrhizal plants may suffer less damage from parasitic nematodes and root pathogens (Marx, 1973; Schenk et al., 1975). Effects of mycorrhizae can vary with insect species, mode of feeding and environmental conditions (Pacovsky et al., 1985; Lin and Kogan, 1990; Gange and West, 1994). For example, Borowicz (1997) found that mycorrhizal colonization of soybean improved performance of Mexican bean beetle larvae, but only under low P concentrations.

N-fixing symbioses can also affect plant-herbivore interactions. Over 15,000 species in at least 12 plant families participate in N-fixing symbioses (Akkermans and van Dijk, 1981). Increased N availability may allow greater production of N-based defensive compounds such as alkaloids (Janzen, 1981; Johnson and Bentley, 1991). Fixed N taken up by other plants may also enhance their production of N-based defensive compounds (Holah and Clay, unpublished data). However, as in the case of mycorrhizas, improved host nutrition could possibly also result in greater attractiveness to herbivores (Wilson and Stinner, 1984), and herbivory may reduce the plant’s ability to support the bacterial symbionts. Further, N obtained through N-fixation versus uptake through the root system may have different characteristics and temporal patterns of availability (Johnson and Bentley, 1991).

The effects of grass-endophyte symbiosis on herbivores are well known. Most endophytes produce N-rich alkaloid compounds that deter or poison a range of vertebrate and invertebrate herbivores (Bush et al., 1997; Clay, 1997). Endophyte-infected tall fescue and perennial ryegrass cause a variety of problems in domestic livestock as a result of fungal alkaloids (Clay, 1988). Infected plants may sustain less damage than uninfected conspecifics or other grasses if herbivores avoid them. Organisms as diverse as aphids, dark-eyed juncos and cattle can distinguish between infected and uninfected tall fescue (Latch et al., 1985a; Madej and Clay, 1991; Van Santen, 1992). Avoidance or reduced performance may result in decreased herbivore population sizes (Cheplick and Clay, 1988; Giuliano et al., 1994; Coley et al., 1995; Fortier et al., 2000). However, Saikkonen et al. (1998) suggested that there is considerable variation among plant/fungal combinations in their effects on herbivores, perhaps reflecting variation in numbers and concentrations of alkaloid compounds produced by different grass-endophyte combinations (Siegel et al., 1990).

There is also evidence of indirect effect of endophyte infection on predators mediated through herbivores. For example, Bultman et al. (1997) found that pupal mass of two parasitoid species was significantly reduced when their host lepidopteran, *Spodoptera frugiperda*, was reared on endophyte-infected versus uninfected tall fescue. In a more recent study, Omacini et al. (2001) found that endophyte infection of Italian ryegrass (*Lolium multiflorum*) had a large, indirect effect on parasitoid communities of aphids. Relative to endophyte-infected plant populations, uninfected *Lolium* plants supported higher densities of aphids that had a significantly higher rate of parasitism. Moreover, the parasitoid community was larger and more diverse on uninfected plants. A generalist hyperparasitoid also occurred with endophyte-free plants but not with infected plants. They interpret these results as an endophyte-induced reduction in food quality and therefore energy transfer, rather than reduced productivity. Together these studies illustrate a trophic cascade of an unusual sort where the microbial symbiont directly alters host plant chemistry and palatability, thereby indirectly affecting herbivores and their predators.
TALL FESCUE, ENDOPHYTE SYMBIOSIS AND PLANT COMMUNITY STRUCTURE: A CASE STUDY

A series of long-term field experiments at Indiana University are examining the effects of endophyte symbiosis with tall fescue on diversity and productivity of successional grassland plots. Because the fungus is seed-borne and does not spread contagiously, field experiments offer the opportunity to examine how endophyte symbiosis affects both the plant community and herbivores of that community.

The symbiotic interaction between tall fescue and the fungal endophyte Neotyphodium coenophialum is the best-studied endophyte interaction, although it may not be typical of all grass-endophyte interactions (Clay, 1998; Saikkonen, 1998). Tall fescue was introduced from Europe and is now the most widespread perennial cool-season grass over much of the United States. It is widely planted for forage, turf and soil conservation, but also occurs in a variety of unmanaged habitats (Ball et al., 1993; Hiebert, 1990). Endophyte-infected plants are generally more vigorous and persistent than co-occurring endophyte-free plants, and are more resistant to herbivory (Clay, 1997).

In fall 1994 a large mowed field of perennial grasses and forbs at the Indiana University Botany Experimental Farm was thoroughly plowed and disked. Eight 20 m x 20 m plots were alternately seeded with either infected or uninfected tall fescue seed at a uniform rate. Dense stands of tall fescue resulted in all plots along with a diverse assemblage of plant species germinating from dispersed seed, the seed bank, or by regeneration from vegetative fragments. No other treatments (chemical pesticides, herbicides, mowing etc.) were applied. Repeated sampling has indicated that the frequency of endophyte infection of tall fescue in uninfected plots was less than 5% whereas in infected plots infection frequency was >95%. Twenty random quadrats (0.5 m x 0.5 m) were harvested from each plot in June and October each year and vegetation samples were sorted by species or into thatch, dried and weighed. Prairie voles (Microtus ochrogaster) appear to be the major source of herbivory in the experimental plots. Voles characteristically cut tillers off near ground level at an oblique angle and trim fleshy tiller bases into 4–6 cm lengths, which were found in abundance. In 1996 and 1997 voles were trapped in multiple-capture live traps to estimate population sizes, sex ratios and reproductive status (see Fortier et al., 2000). Trapping was done weekly May through July by setting traps in the afternoon and checking them the following two mornings. Captured animals were toe-clipped for future identification, weighed, sexed and their reproductive condition assessed before release at the same location.

Initially the composition of vegetation did not differ between the two types of plots and there was no significant effect of infection status on any variable measured (Clay and Holah, 1999). However, since the first year of the study characteristics of the vegetation have diverged between infected and uninfected plots. Mean species number per quadrat fluctuated from spring to fall harvests but has gradually diverged such that at the final three harvests mean values for uninfected plots were only 60% that of uninfected plots (Fig. 3). Thus, the presence of the endophyte suppressed species richness relative to uninfected plots. Six species present in uninfected plots, such as clover (Trifolium repens) and box elder (Acer ne-
**gundo**, were not found in infected plots. This is consistent with the hypothesis that vole feeding affects plant species composition given that voles are known to selectively graze forbs and tree seedlings (Linnroth and Batzli, 1984; Ostfeld et al., 1997; Howe and Brown, 1999).

Despite differences in diversity, total productivity was similar between plot types (Fig. 4). Standing biomass varied from 300 to 800 g/m² and declined over the course of the study as litter biomass increased. However, biomass composition did differ significantly between infected and uninfected plots (Fig. 5). Tall fescue was significantly more abundant in infected plots relative to uninfected plots while other perennial grasses and forbs were less abundant. At the final harvest, mean biomass of tall fescue in infected plots was 321 ± 17 g/m², compared to 179 ± 14 g/m² in uninfected plots, an 80% increase. Thus, infected plots became near monocultures of tall fescue while uninfected plots became more diverse mixtures of other grasses and forbs, demonstrating that this symbiotic microorganism can significantly enhance the productivity of its host and modify the structure of plant communities.

A number of possible mechanisms could explain these observed differences. It should be emphasized however that the primary goal of this study was to quantify the consequences of endophyte infection on community structure, and not to evaluate alternative mechanisms. Nevertheless, three potential mechanisms suggest themselves based on results from other studies. The increased dominance of tall fescue in endophyte-infected plots may reflect their greater interspecific competitive ability, particularly against other perennial grasses which were more abundant in uninfected plots (Fig. 5). Multiple studies have demonstrated that endophyte-infected tall fescue is more productive and competitive than uninfected fescue under a wide range of circumstances (Clay et al., 1993; Shelby and Dalrymple, 1993; Clay, 1997). A second potential mechanism is enhanced stress tolerance of endophyte-infected tall fescue, particularly drought stress (West et al., 1993; Hill et al., 1996). 1997 and 1998 both had extended periods of severe drought conditions in southern Indiana. Loss of cover of tall fescue in uninfected plots may have allowed increases of other perennial grasses and higher levels of plant species richness.

A third possible mechanism is decreased herbivory of tall fescue in infected plots by voles and other herbivorous animals. Field observations suggested that vole herbivory was the major type of tall fescue herbivory and therefore a likely contributor to the changes in vegetation. Other studies have found that small mammal populations are
less abundant in endophyte-infected tall fescue stands (Coley et al., 1995) and that prairie voles exhibited decreased growth rates and reproductive success on a diet of endophyte-infected versus uninfected tall fescue seed (Durham and Tannenbaum, 1998). Conover (1998) found that meadow voles (Microtus pennsylvanicus) suffered increased mortality at high temperatures when fed leaves and stems of infected tall fescue. Giuliano et al. (1994) suggested that the long-term decline of eastern cottontail rabbits (Sylvilagus floridanus) in Kentucky reflects the great increase in abundance of tall fescue over the past several decades. Based on the analysis of rabbit stomach contents, they found that tall fescue was the most utilized food plant by rabbits. The majority of tall fescue in Kentucky is endophyte-infected (Giuliano et al., 1994). In general, endophyte-infected tall fescue has detrimental effects on the survival, growth and/or reproduction of a wide range of vertebrate species (Latch, 1993).

In our study plots both prairie (M. ochrogaster) and meadow voles were present but the former occurred at much higher frequencies. Over two field seasons nearly 750 voles were trapped at least once. We used mark-recapture analysis programs provided by C. J. Krebs to determine reproductive condition, population density, sex ratio and growth rates. Estimated population sizes increased over the season in 1996 and continued to increase in 1997, reaching a peak of about 40 animals per plot in late spring (Fig. 6). There were no significant differences in total density between infected and uninfected plots either year.

In 1996, when vole densities were lower, there was a significantly greater proportion of males on infected (58%) versus uninfected (42%) plots (Fortier et al., 2000). However, in 1997, when densities were higher, sex ratios were equivalent between plot types. Interestingly, there were relatively large shifts in species richness and biomass composition between 1996 and 1997 (Figs. 3 and 5) at the same time vole population densities were increasing. Reproduction of male voles was unaffected by the endophyte with all males greater than 22 g being reproductive. However, female voles were sexually mature at lower weights in uninfected plots in 1997, when densities were high, but not in 1996 (Fig. 7), indicating a significant negative effect of population density on female sexual maturity in infected plots. Varney et al. (1991) also found that a diet of endophyte-infected tall fescue seed delayed the onset of puberty in female mice, although Tannenbaum et al. (1998) did not find any difference between infected and uninfected tall fescue seed in suppressing white-footed mouse reproduction.

Our results our consistent with the idea that at low population densities, female voles (but not males) avoid endophyte-infected tall fescue. As population density increased, sex ratios became equal in both infected and uninfected plots. There were no barriers to dispersal between plots. Female voles may disperse from uninfected to infected plots where feeding on tall fescue inhibits their reproductive capacity as competition for food and territory becomes more severe. Ultimately, this grass-endophyte-herbivore interaction could result in smaller vole populations relative to potential population sizes in endophyte-free grasslands. From the plant’s perspective, under normal conditions female voles may avoid infected tall fescue and feed instead.
on other grasses and forbs. In contrast, uninfected tall fescue is an attractive food plant and is grazed heavily. The greater damage to tall fescue in uninfected plots may reduce its relative competitive ability against less heavily grazed grasses and so it declines in abundance. In infected plots, other perennial grasses and forbs are grazed more heavily and so decrease in competition with tall fescue.

The proposed interactions suggest that the endophyte may be a potent regulator of both primary producer and herbivore trophic levels. Host plant productivity is directly increased by infection and may be indirectly facilitated by differential grazing of infected versus uninfected plants. Herbivores, and, by extension, predators of those herbivores, are inhibited by endophyte toxins and their feeding patterns help drive compositional changes in plant communities. The proposed role of herbivores is supported by much research with tall fescue showing preference for uninfected plants (Latch et al., 1985b; Madej and Clay, 1991; Van Santen, 1992), reduced animal performance on infected plants (Cheplick and Clay, 1988), and reduced population sizes where endophyte infected tall fescue is common (Giuliano et al., 1994; Coley et al., 1995). This hypothesis predicts that differences in vegetation between infected and uninfected plots would be reduced or eliminated if voles and other herbivores were excluded from the system. A definitive test of the hypothesis requires field experiments where both vole herbivory and endophyte infection are controlled and where both vegetation and vole responses are measured.

CONCLUSIONS

I have argued here that microbial symbionts of plants are important regulators of community structure through their effects on resource acquisition and growth of primary producers, and through their effects on herbivores. Microbial symbionts may represent keystone mutualists affecting both higher and lower trophic levels (see also Terborgh, 1986). The notion of “inside-out” regulation of communities is appropriate in light of both the intermediate trophic position (i.e., primary consumers) and physical location of these symbionts inside of plants.

Symbiotic regulation of communities may be a more general phenomenon than we realize. The relative lack of data on this topic is partly due to difficulties in manipulating microbial symbioses in the field compared to the relatively easy methods of caging to exclude predators or fertilizing to increase resources. It is also partly due to the tendency to view microbial symbionts of plants as part of the plant itself. This is a mistake I believe, and has lead to an incomplete view of the factors that regulate terrestrial communities. Very small organisms can have very large effects on ecological systems.
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