Biological Interactions in Grassland Soils and Productivity

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BIOLOGICAL INTERACTIONS IN GRASSLAND SOILS AND PRODUCTIVITY

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Abstract

This paper describes research on interactions between grassland plant species and soil microorganisms. Both parasitic and symbiotic microorganisms modify nutrient transfers between plants and soil. Experiments are described in which nematode infection of clover increased nitrogen transfer to companion ryegrass plants. Infection of clover enhanced activity of soil bacterial and fungal communities. Legume genotypes differing only in responses to symbionts (rhizobium and arbuscular mycorrhizal fungi) and pathogens are being developed for studies of gene expression during establishing and functional symbioses. Such plants can be used in experiments as defined perturbations that will provide information on the interactions and functions of symbiotic and pathogenic microorganisms. Such studies, related to field observations, may have value for defining biological attributes of sustainable grassland soil systems.

Keywords: Gene expression, nematodes, nutrient cycling, mycorrhizae, rhizobia, white clover.

Introduction

Soils under temperate grasslands develop rich biological communities of micro-flora and fauna (Bardgett and Cook, 1998) that determine soil fertility and hence productivity of grassland systems. The roles of some of the soil biota are well known particularly as direct interactions with single crop plant species. For example, root-feeding nematode species reduce plant growth and crop yield. In contrast, symbioses with arbuscular mycorhizal fungi (AMF) and with rhizobia contribute directly to plant phosphorus and nitrogen nutrition. There are also indirect effects of these interactions. For example, nematodes impact on microbial activities, affecting decomposition and plant growth, and AMF promote plant health, through alleviation of stress. Analysis of biological communities and study of their interactions with each other and with plants is important to increase understanding of soil functions.

We describe our current approaches to the study of 1) how nutrient cycling is affected by grassland nematodes and 2) plant microbial interactions with legumes to identify the genetic basis of effective symbioses.

Materials and methods

Root nematodes and nitrogen transfer. Experiments to assess nematode impacts on nutrient cycling used white clover infected by clover cyst nematode, *Heterodera trifolii*. Clover and perennial ryegrass, *Lolium perenne*, were grown in field soil microcosms in controlled environments. In half the microcosms, soil was infested by addition of infective juvenile nematodes. In one experiment, expanded leaves of clover plants were immersed in a solution of $K^{15}NO_3$ for 72h and the distribution of ^{15}N to the companion ryegrass was measured. After 12 weeks, when second generation nematodes were feeding on the clover roots, root biomass was determined and active soil microbial biomass estimated by chloroform fumigation-extraction and by analyses of phospholipid fatty acids (PLFA) (Bardgett *et al*., 1999a, b).

Genetic basis of effective symbioses. Two legumes are used to study gene expression in symbiotic interactions: a model legume, *Lotus japonicus,* and the agriculturally important, white clover. One genetically tagged transformant of *L. japonicus* (line T90) responds to the presence of the nitrogen-fixing bacterium, *Mesorhizobium loti*, by expressing the reporter gene, â-glucuronidase (GUS), in roots and nodules. This tagged gene has homology to genes encoding calcium-binding proteins (Webb, Skøt *et al*., 2000). Homozygous seedlings from T90 were challenged with two AMF species, *Glomus intraradices* and *G. mossae* and with pathogenic, root-knot nematode, *Meloidogyne hapla.* We monitored expression of the rhizobium-induced promoter by histochemically staining for GUS expression in inoculated roots.

Traits of agronomic importance are fixed in near-isogenic lines (NILs) of white clover that have been uniquely developed by IGER (Michaelson-Yeates et al, 1997). 43 NILs were infected with *G. mossae* and plant growth responses and AMF root infection monitored.

Results and Discussion

Root nematodes and nitrogen transfer. Root biomass of nematode-infected white clover plants was 140% more than that of uninfected controls. There were 37% more *Rhizobium*-nodules on these roots and enhanced nitrogen fixation capacity and nitrogen supply to the plant may account for the heavier roots of infected plants. Nematode infection of white clover roots also led to a significant increase (217%) in root biomass of the companion ryegrass, not itself infected by the nematodes, and to a 322% increase in grass uptake of white clover-derived ^{15}N. Total abundance of PLFA, a measure of the 'active' microbial biomass, in the rhizosphere soil from lightly infested plants was twice that in soil from uninfected controls. Increases in the size of the active rhizosphere microbial biomass were associated with increases in the abundance of fatty acids synthesised by gram-negative and gram-positive bacteria, and fatty acids specific to fungi.

These experiments suggest that non-damaging infection of white clover roots enhances N flux into the soil, where it is recycled by the microbial community and taken up by neighbouring ryegrass. This is evidence of a linkage between nematode-induced increases in soil microbial metabolism and below ground performance of companion plants. Insect root herbivory also increases loss of nitrogen from clover roots (Murray and Hatch, 1994). It has also been shown that low densities root infection of white clover by cyst nematode increased rhizodeposition of carbon (Yeates et al., 1998). The overall effect of such interactions is to increase root growth of both the host plant (white clover) and also the uninfected neighbouring grass, presumably influencing productivity and competitive interactions between these plant species (Bardgett et al., 1999b).

These experiments emphasise the importance of microorganisms in nutrient transfers within grassland soils. It seems likely that rhizodepositions of carbon will be important energy sources for nutrient transfers facilitated by microorganisms. Observations on the changes in, for example, nematode and fungal communities in response to changes in management practices (mainly quantity and quality of nitrogen supply) indicate that fungal activity is more prominent in lower input systems (Yeates et al., 1997).

Genetic basis of effective symbioses. In *L. japonicus* line T90, the GUS-tagged promoter was induced by symbionts - *M. loti* and both species of AMF - but not by root-knot nematodes (Tuck *et al*., 2000). Further tests of the specificity of this tagged gene include challenge by a range of other pathogens, such as root-rot fungus, *Fusarium* sp. and *Rhizoctonia* sp. and chemicals, such as Nod factor signals.

Clover genotype influenced both plant response to AMF as well as AMF infection rates, supporting previous findings (Manske, 1990). Infection rates ranged from 0 to 37%, with shoot weight increasing with infection of some plants but decreasing for others. Lines that were closely related (i.e. derived from the same original parent line) but that differed in responses to infection were selected for more detailed study. Nutrient flowing culture microcosm systems are being used to relate key physiological markers (such as phosphate uptake) to plant gene expression using differential display analysis. Other white clover NILs have been identified that differ in response to rhizobium and to a nematode pest, *Ditylenchus dipsaci*.

Plants, such as the single gene transformed *Lotus* plants and clover NILs that have been characterised genetically and phenotypically, are valuable for making controlled perturbations in realistic grassland soil systems. Growing such contrasting plants and comparing the responses of the soil biota and consequences for plant nutrition and soil function will contribute to understanding how complex nutrient cycles may be regulated by interactions between heterotrophic micro-organisms. Such understanding will be of value in designing and managing sustainable grassland systems.

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