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Impact of root herbivory by insect larvae on soil microbial communities

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Abstract − Bentgrass (*Agrostis capillaris*) and clover (*Trifolium repens*) were grown as pure swards and mixtures in pots containing soil from the NERC Soil Biodiversity field site located in Scotland. Six weeks after plant establishment leatherjacket larvae (*Tipula paludosa*) were added at field density to half the pots and the impacts of their feeding on plant shoot and root biomass and soil microbial communities was determined after 10 days. Plate counts and community level physiological profiles (CLPP) were used to characterise the microbial communities. Larval herbivory had a significant negative effect on shoot growth of both grass and clover and root biomass of grass. In mixed swards, larvae preferentially fed on clover. Soil microbial community structure was altered in the presence of larvae with populations of pseudomonads being significantly increased. These community differences may be attributed to increased quantity and qualitative changes in carbon flux to the soil as a result of root herbivory, as indicated by differences in the CLPPs of microbial communities in the presence and absence of larvae. This was mainly due to increased utilisation of some sugars, carboxylic and amino acids in the presence of larvae. © 2001 Éditions scientifiques et médicales Elsevier SAS

Biolog / community level physiological profiles / grasses / insect root herbivores / soil microorganisms

1. INTRODUCTION

The increasing emphasis on low input extensive agriculture means that achieving sustainable plant growth will depend on devising strategies to maximise the use of soil nutrient resources. In such systems, which include upland grasslands, the soil microbial biomass is critical in regulating soil ecosystem level processes, such as nutrient cycling [12]. Therefore, it is vital to understand the factors that influence its structure and activity. Root herbivory by insect larvae will change soil carbon and nutrient flows and is, therefore, likely to have a major impact on soil microbial communities and subsequently nutrient cycling. However, despite the importance of microbial processes in these systems, few studies have linked them to root herbivory. The root feeding leatherjacket larvae (*Tipula paludosa*) are important pests of grasslands and cereal crops [5]. Although much work has been done on the impact of these larvae on yield reduction [1, 2, 14], the impact on microbial communities has not been evaluated.

Community level physiological profiles (CLPP) (Biolog®) have shown their potential as an ecologically relevant method to characterise microbial communities from the rhizosphere of different plant species [7, 9, 10]. The technique measures utilisation of a variety of carbon compounds and is therefore a meaningful assay of communities because carbon is a major factor governing microbial growth in soil [17].

The aim of this study was to determine the impact of root herbivory by *T. paludosa* on microbial community structure in the rhizosphere of *Agrostis capillaris* and *Trifolium repens*, using selective plating techniques and CLPP. The hypothesis to be tested was that larval root herbivory would change the microbial community as a consequence of increased root detritus and exudation and this would be reflected in a difference in CLPP between these communities.

2. MATERIALS AND METHODS

2.1. Experimental design

Soil (Sourhope series; brown podzol; Tot C 101 $g \cdot kg^{-1}$, Tot N 7.7 $g \cdot kg^{-1}$) was collected from a field of permanent grassland at Sourhope Research Station (55°28'30"N/2°14'W) located in the Borders of Scotland. The soil was sieved (< 6 mm) then used to fill 23 cm diameter pots to a depth of 20 cm. Pots were sown with seeds of either *A. capillaris* (bentgrass), *T.*

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repens (white clover) or a mixture of both at a density of 25 $g \cdot m^{-2}$. Six replicates of each treatment were grown in the greenhouse until sward establishment (6 weeks) and watered daily to excess. After 6 weeks 20 *T. paludosa* (Meigen, 1830) larvae (fourth instar), collected from the same field site, were added to each of three replicate pots of each sward type (density 482 $larvae \cdot m^{-2}$). The pots were harvested 10 days after introduction of larvae.

2.2. Plant biomass analyses

Shoots were removed by clipping at soil level and shoots of *A. capillaris* and *T. repens* were separated from the mixed swards. Roots were removed by dry sieving and manual removal. Roots from mixed swards could not be separated into species due to their similarities. Shoots and roots were weighed, oven dried at 80°C for 48 h and reweighed.

2.3. Microbial community analyses

Microbial communities were extracted from soil by shaking 10 g of soil in 100 mL of ¼ strength Ringers solution (Oxoid) for 10 min, on a wrist action shaker. After ten-fold serial dilution in Ringers, suspensions (0.1 mL) were spread in duplicate on the following media. Tryptone Soy agar (1/10 strength, Oxoid) plus cycloheximide (50 $mg \cdot L^{-1}$) to enumerate bacteria, *Pseudomonas* isolation agar (Oxoid) selective for pseudomonads and Czapek-Dox agar (Oxoid) plus ampicillin (10 $mg \cdot L^{-1}$), streptomycin and tetracycline (50 $mg \cdot L^{-1}$) for enumeration of fungi. Plates were incubated at 25°C and colonies counted weekly until no new growth appeared.

Biolog® GN microplates (Biolog Inc., Hayward, CA, USA), which contain 95 different carbon sources, were used together with exudate profile microplates, prepared using Biolog® MT plates, [3] to construct community level physiological profiles (CLPP) of the microbial communities. A 50 mL aliquot of the 10^{-4} dilution of the same rhizosphere soil samples used in the enumeration of culturable microorganisms was centrifuged at 2000 rpm for 10 min to separate soil and minimise addition of soil or root derived carbon into the system. A 0.15 mL aliquot of each sample was dispensed into each well of the GN and exudate plates. The plates were incubated at 15°C for 5 days and colour development (carbon utilisation) measured as absorbance at 590 nm every 24 h using a microplate reader (Vmax, Molecular Devices, Oxford, UK).

2.4. Statistical analyses

All plant biomass data was untransformed, with the exception of the shoot data from the mixed swards that was square root transformed, prior to ANOVA (Genstat 5.4, NAG Ltd., Oxford, UK). Microbial population data was log transformed prior to ANOVA. For the CLPP data the average well colour development (AWCD) of all 125 carbon sources for each sample were calculated and used to transform individual well values to eliminate variation in well colour development caused by different cell densities [6, 8]. The AWCD of different substrate groups (sugars, oligosaccharides, carboxylic acids, acidic amino acids, basic amino acids, neutral amino acids, amides, phenolic acids, alcohols, N-heterocyclic-N, long chain aliphatic acids and all compounds) was calculated prior to performing ANOVA. The CLPP data from each incubation time were also analysed by canonical variate analysis (CVA) (Genstat 5.3) to differentiate samples based on their overall patterns of carbon utilisation and to identify the carbon sources most responsible for the discrimination.

3. RESULTS

3.1. Plant biomass

In the single species pots larvae significantly ($F < 0.001$, 2 d.f.) reduced the shoot biomass of both *A. capillaris* and *T. repens* (*table I*). In the mixed sward only, *T. repens* shoot biomass was significantly reduced in the presence of larvae ($F = 0.028$)(*table I*). The root biomass of both species was reduced in pots with larvae, but this was only significant ($F < 0.001$) for *A. capillaris* in the single species pots (*table I*).

3.2. Microbial communities

Numbers of total bacteria tended to be increased in the presence of larvae in pots sown with *A. capillaris* and the *A. capillaris/T. repens* mixture, although these increases were not significant. Numbers of pseudomonads, however, were significantly ($F < 0.001$, 2 d.f.) increased in all treatments in the presence of larvae (*figure 1*). Populations of yeasts and fungi were not significantly affected by any treatment (data not

Table I. Shoot and root biomass of *Agrostis capillaris* and *Trifolium repens* after10 days in the presence and absence of *Tipula paludosa* larvae.

Plant	Shoot biomass $g \cdot pot^{-1}$	
	- larvae	+larvae
Agrostis capillaris (alone)	3.31^{c}	1.79^{b}
Trifolium repens (alone)	1.64^{b}	0.04^{a}
Agrostis capillaris (mixture)	3.02^{c}	2.42^{c}
Trifolium repens (mixture)	0.44^{b}	0.12^{a}
Plant	Root biomass $g \cdot pot^{-1}$	
	- larvae	+ larvae
Agrostis capillaris	1.92^{b}	0.96^{c}
Trifolium repens	0.31^{a}	0.17^{a}
Agrostis capillaris & *Trifolium repens*	1.26^{b}	1.03^{b}

Values are means of 3 replicate samples (2 d.f). Values followed by the same letter are not significantly different ($P < 0.05$).

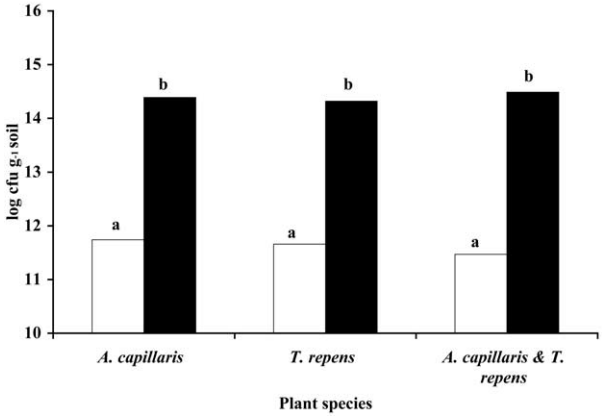

Figure 1. Populations of pseudomonads in the rhizosphere of *Agrostis capillaris*, *Trifolium repens* and *A. capillaris/T. repens* mixtures in the presence and absence of *Tipula paludosa*. (Values are the means of three replicate samples. Bars with the same letter are not significantly different $P < 0.05$).

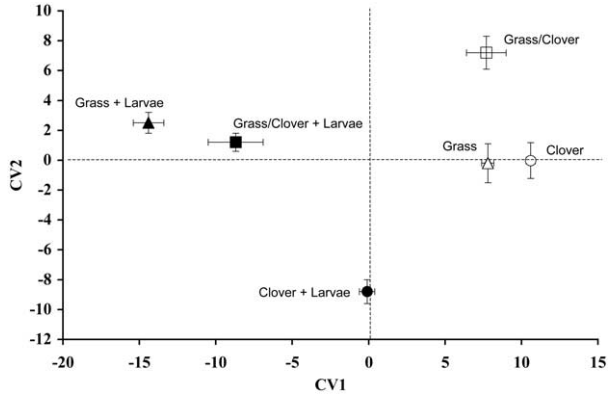

Figure 2. Canonical variate scores of soil samples based on CLPP of microbial communities from the rhizosphere of *Agrostis capillaris* (▲), *Trifolium repens* (●) and *A. capillaris/T. repens* (■) mixtures in the presence (solid symbols) and absence (open symbols) of *Tipula paludosa*. Values are for 72 h incubation. Bars represent standard deviation.

shown). The CLPP of the microbial communities showed that, when comparing pots containing the same plant species, microorganisms from pots containing larvae had significantly greater utilisation of all groups of carbon compounds, except for phenolic and long chain aliphatic acids, than those from the same swards without larvae (*table II*). CVA results presented are those after 72 h of incubation because this gave the best discrimination amongst treatments. CVA clearly differentiated the samples from pots with and without larvae on canonical variate (CV) 1 (*figure 2*). Analysis of the loadings of the carbon sources on this CV indicated that in the presence of larvae some sugars (arabinose, galactose, maltose), carboxylic (tartaric, malic, oxalic) and amino acids (lysine, serine) were mainly responsible for the discrimination.

4. DISCUSSION

Herbivory by *T. paludosa* significantly reduced the shoot biomass of both *A. capillaris* and *T. repens* and the root biomass of *A. capillaris*, when grown in monocultures. In mixed swards *T. paludosa* herbivory only reduced *T. repens* shoot biomass, suggesting *A. capillaris* may be less nutritious to *T. paludosa*. Ramsell et al [15] showed that *T. paludosa* larvae preferred to graze on *Lolium perenne* than *Rumex obtusifolius*, significantly reducing *L. perenne* root biomass in monocultures and mixtures. However, grazed *L. perenne* was found to be a stronger competitor of *R. obtusifolius*, due to enhanced shoot growth rates [15]. These studies suggest that differential shoot and root grazing in mixtures will have important

Table II. Average well colour development (utilisation) of the main groups of carbon compounds in the Biolog GN and exudate plates by microbial communities from the rhizosphere of *Agrostis capillaris* and *Trifolium repens* in the presence and absence of *Tipula paludosa* larvae.

Carbon compounds	*A. capillaris*	*T. repens* - larvae	*A. capillaris* & *T. repens*	*A. capillaris*	*T. repens* + larvae	*A. capillaris* & *T. repens*	F values
All	0.091^{a}	0.094^{a}	0.074^{a}	0.284^{b}	0.283^{b}	0.248^{b}	0.003
Sugars	0.042^{a}	0.066^{a}	0.039^{a}	0.362^{b}	0.324^{b}	0.272^{b}	< 0.001
Oligosaccharides	0.045^{a}	0.050^{a}	0.045^{a}	0.442^{b}	0.432^{b}	0.337^{b}	< 0.001
Alcohols	0.079^{a}	0.083^{a}	0.090^{a}	0.385^{b}	0.358^{b}	0.273^{ab}	0.004
Carboxylic acids	0.125^{ab}	0.122^{ab}	0.096^{a}	0.286^{c}	0.293^{c}	0.240^{b}	0.022
Acidic amino acids	0.115^{a}	0.142^{a}	0.133^{a}	0.434^{b}	0.424^{b}	0.393^{b}	0.012
Basic amino acids	0.154^{abc}	0.134^{ab}	0.091^{a}	0.300^{bc}	0.340^{c}	0.307^{bc}	0.034
Neutral amino acids	0.075^{a}	0.074^{a}	0.044^{a}	0.286^{b}	0.245^{b}	0.263^{b}	0.003
Heterocyclic N	0.079^{a}	0.138^{ab}	0.078^{a}	0.328^{bc}	0.377^{c}	0.297^{bc}	0.013
Amides	0.030^{a}	0.047^{ab}	0.041^{ab}	0.116^{bc}	0.146^{c}	0.151^{c}	0.015
Phenolics	0.100^{ab}	0.095^{ab}	0.074^{a}	0.125^{ab}	0.136^{b}	0.147^{b}	0.093
Long chain aliphatics	0.108^{a}	0.100^{a}	0.099^{a}	0.147^{a}	0.154^{a}	0.163^{a}	0.146

Values are means of 3 replicate samples. Values followed by the same letter are not significantly different.

consequences for plant competition and community composition in grasslands.

Soil microbial community structure and function was altered in the presence of larvae with significantly higher populations of pseudomonads and a change in metabolic profiles of the microbial communities from pots with *T. paludosa*. Denton et al. [4] showed that root herbivory by clover cyst nematodes significantly increased soil microbial biomass. They hypothesised that nematode herbivory increased root exudation, as shown by Yeates et al [18], although they found no change in microbial community structure. Increased root exudation tends to increase bacterial abundance in soil [10, 11]. The increase in *Pseudomonas* species, in this study, is not surprising as they are a nutritionally diverse group of bacteria, have a higher growth rate in soil than other species and there is increasing evidence of their selective stimulation in the rhizosphere of a range of plant species [10, 13]. The results from this study suggest that root herbivory by *T. paludosa* may increase root exudation and change the character of compounds released because the metabolic profiles of the microorganisms from these treatments were different. In addition, release of larval excrement may also affect the microbial communities, leading to a change in their metabolic profiles. Changes in CLPP due to larvae were mainly due to differences in sugars, carboxylic and amino acid usage. This suggests larval herbivory may increase release of these compounds from roots, or alternately, they may be contained in larval excrement, which then selects for microorganisms capable of utilising these substrates. Studies are currently being undertaken to characterise root exudates from pasture plants subject to *T. paludosa* herbivory [16]. In addition, increased detrital input to the soil, as a result of root severance will impact on the microbial community. This data clearly shows that belowground herbivory changes the structure of the soil microbial community. These changes are likely to impact on nutrient cycling and availability, and therefore have important consequences for pasture management and productivity. Future research will investigate the mechanisms involved in these responses.

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