Available online at www.sciencedirect.com
SCIENCE DIRECT®
Applied Soil Ecology 25 (2004) 51–61
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Impact of *Tipula paludosa* larvae on plant growth and the soil microbial community

Lorna A. Dawson [a,*], Susan J. Grayston [a], Philip J. Murray [b], Jasmine M. Ross [a], Eileen J. Reid [a], Amy M. Treonis [c]

[a] *Macaulay Land Use Research Institute, Environmental Science Group, Craigiebuckler, Aberdeen AB15 8QH, UK*
[b] *Institute of Grassland and Environmental Research, North Wyke, Okehampton EX20 2SB, Devon, UK*
[c] *Department of Biology, Creighton University, 2500 California Plaza, Omaha, NE 68178, USA*

Received 19 September 2002; received in revised form 16 July 2003; accepted 18 July 2003

Abstract

An experiment was performed to study the effect of feeding by the larvae of *Tipula paludosa* (Meig.) on the plant biomass of two contrasting plant species and on the soil microbial community, under controlled conditions. *Agrostis capillaris* (L.) (bentgrass) and *Trifolium repens* (L.) (white clover), were grown in pots, in monoculture and as mixtures, containing soil from an upland grassland site in the UK. After plant establishment, larvae were added to half the pots at field density (480 larvae m^{-2}). After 12 days, the pots were destructively harvested and the shoot biomass, root biomass and the soil microbial community (using plate counts and community level physiological profiles (CLPP)) were assessed. The presence of larvae significantly reduced shoot biomass in *T. repens* growing as monoculture. In pots containing a mixture of *A. capillaris* and *T. repens*, only the shoot biomass of the *T. repens* was significantly reduced. In the single species pots, the larvae significantly reduced the root biomass of both species. The soil microbial community structure changed in the presence of larvae resulting in a significant 10-fold increase in numbers of *Pseudomonas* spp. in the soil. Canonical variate analysis of the CLPP data also showed that microbial communities from the soils with larvae present had a greater utilisation of a number of sugars, amino acids and carboxylic acids. These changes may have arisen as a result of an increase in carbon exudation due to root severance or shoot herbivory, an increase in dead roots or due to larval decomposition or defecation.

Keywords: Clover; Biolog; Grass; Insect larvae; Roots; *Tipula*

1. Introduction

Pasture species are, in general, well adapted to cope with the removal of foliage by above-ground herbivores due to, for example, their meristematic zones being located close to the soil surface, and the ability of the grass plant to store and utilise carbon and nitrogen for regrowth following defoliation (Murray and Clements, 1999). However, the ability of the plant to cope with below-ground herbivory is less well defined. Indeed there are only limited data available on the effects of root herbivory on growth and development of the pasture plant (e.g. Murray et al., 2002). Larval feeding can reduce root biomass by up to 50% (Murray and Clements, 1992). In pastures, the perpetual cover and supply of plant material both above- and below-ground supplies an abundant food supply for the insect community. This, together with the lack

* Corresponding author. Tel.: +44-1224-498200; fax: +44-1224-311556.
E-mail address: l.dawson@macaulay.ac.uk (L.A. Dawson).

0929-1393/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0929-1393(03)00099-4

of soil disturbance through, for example, ploughing, allows large populations of invertebrates to survive (Clements et al., 1990). Of the root-feeders commonly found in grassland, the larvae of the cranefly, *Tipula* spp. (Diptera, Tipulidae), are some of the most important pests of grassland and can occur at densities up to 620 m^{-2} (French et al., 1990). The biomass of invertebrates in permanent pasture can even exceed that of the livestock grazing it (Coulson and Butterfield, 1978).

Numbers of tipulids may be controlled by the application of a pesticide (Mowatt and Jess, 1986; French et al., 1990), although the use of pesticide is only viable when larval numbers are above a threshold (French et al., 1990). Nevertheless, considerable damage can be done at low densities (Blackshaw, 1984). Most studies of *Tipula* spp. to date have concentrated on their effects in lowland pastures (Blackshaw, 1984; Blackshaw and Newbold, 1987; French, 1969; Newbold, 1981), and few investigations have been done on upland pastures (French et al., 1990).

In studies where natural levels of root herbivory were manipulated using insecticides, root-feeders were shown to have a considerable impact on the dynamics of the plant community (Brown and Gange, 1989, 1991). Cover abundance and plant species richness were shown to increase when below-ground herbivory was reduced. The larvae of *Tipula* spp. can feed on most grassland species (Clements, 1984), although when given a choice, some preference has been reported (Ramsell et al., 1993; White and French, 1968). *Tipula* spp. can reduce yield through both shoot and root herbivory. Dawson et al. (2002) found that larvae of *T. paludosa* fed voraciously on roots of *Trifolium repens* (L.), causing a reduction in root length of 15% in 7 days, compared to no significant effect on root length in *Lolium perenne* (L.). It is therefore likely that larval grazing would have contrasting effects on different plant species.

In upland grassland, the activity of the soil microbial biomass is a major driver of soil nutrient availability. Therefore, it is important to understand the factors that influence the structure and activity of the soil microbial community. Below- as well as above-ground herbivory can have a part to play. The action of soil invertebrates such as *Tipula* spp. can accelerate the loss of plant material to soil saprophytes, due to severance of the root system, resulting in an increased supply of dead roots, increased root exudation and excretion of faeces. They can, therefore, potentially impact upon the soil microbial community and subsequently nutrient cycling. Despite the importance of the role of microorganisms in grassland, few studies have been linked to larval herbivory. The aim of this experiment was to investigate, under controlled conditions, the impact of the larvae of *T. paludosa* on the shoot and root biomass of two contrasting pasture species, *T. repens* and *Agrostis capillaris* (L.), and the associated soil microbial communities. The hypotheses to be tested were (a) that *T. paludosa* would have contrasting effects on the two plant species and (b) that larval herbivory would change the soil microbial community structure.

2. Material and methods

2.1. Experimental design

Topsoil (to 20 cm depth) was collected from an untreated, unimproved (National Vegetation Classification (NVC)—U4d, Rodwell, 1992) upland grassland site at Sourhope Research Station (55°28′30″N/2°14′W) located in the Scottish Borders. The soil was sieved through a 6 mm mesh and loosely consolidated into 23 cm diameter pots (6530 cm^{-3} in each pot). Pots were sown as monocultures with seeds of *A. capillaris* (common bentgrass), and *T. repens* (white clover cv. kent wild white), and also a (50:50 w/w) mixture of *A. capillaris* and *T. repens* at a standard seed density of 25 g m^{-2} in each pot. A 60 mm soil core was removed from each pot and minirhizotron tubes of 50 mm internal diameter were inserted at an angle of 30° to the vertical and to a depth of 20 cm. The tubes were held with the upper face in contact with the relatively undisturbed soil, while the underside, which was not observed, was back-filled with sieved field soil.

The pots were established in three blocks in a heated glasshouse, each block consisting of six pots with one pot from each species by treatment combination, randomly allocated. A circle of 1 mm mesh was placed in the bottom of each pot, and a 10 cm high ring of 1 mm mesh was placed around the top edge of each pot to prevent escape of larvae. The pots were watered daily to excess with demineralised water and allowed to drain to waste. After germination, the plants were

allowed to grow for 2 weeks in the glasshouse and were then moved to an open sided glass house. Blocking layout was retained throughout the experiment.

Third instar larvae of *T. paludosa* were collected from the field site in May and divided into groups of 20, ensuring that size and weight of larvae in each group was similar. The number of larvae chosen for the experiment was equivalent to maximum densities found in cores taken from the Sourhope field site in the 1998/1999 season (unpublished data). These larvae tend to be found in patches with enormously high numbers of individuals, our aim was to mimic such stress on plants as found in those patches. Six weeks after plant establishment, 20 larvae were added to each of half of the pots by scattering them over the surface giving a density of 480 larvae m^{-2}. Within an hour most of the larvae had burrowed into the soil. The pots were harvested 12 days after the introduction of the larvae.

2.2. Root dynamics

The minirhizotron tubes were allowed to stabilise for 6 weeks before filming began. The images were recorded with a Bartz (Bartz Technology Company, Santa Barbara, CA, USA) high magnification minirhizotron colour camera system at vertical soil depths of 5, 7, and 9 cm, approximately every 2 days. Images were analysed for fine root appearance and disappearance using RooTracker software (Duke University, Durham, NC, USA). The root numbers in all these squares were then converted to a density (assuming that 2 mm depth into the soil was observed) and to a rate of new root appearance and disappearance basis, prior to statistical analysis at each individual date.

2.3. Plant biomass and nutrient analyses

Shoots were removed by clipping at soil level and the shoots of *A. capillaris* and *T. repens* in the mixture pots were separated. The shoots of each species were weighed, oven dried at 80 °C for 48 h and reweighed. Roots were removed from the soil by dry sieving (>500 μm), and manual separation from the soil with tweezers. The roots were weighed and length was measured using a Comair (Commonwealth Aircraft Corporation Ltd., Victoria 3207, Australia) root length scanner, which calculates root length based on an intersection method (Newman, 1996; Tennant, 1975). The roots of the two species in the mixture pots could not be identified or separated accurately and consequently one root sample was measured per pot. After oven drying for 48 h at 80 °C the roots were again weighed, ball milled and analysed for total C and total N using an elemental analyser (Carlo Erba, Milan, Italy).

2.4. Microbial community analyses

2.4.1. Plate counts

Microbial communities were extracted from the soil by shaking 10 g of soil in 100 cm^3 of quarter strength Ringers solution (Oxoid) for 10 min, on a wrist action shaker. After a 10-fold serial dilution in Ringers, suspensions (0.1 cm^3) were spread in duplicate on the following media: Tryptone soy agar (one-tenth strength, Oxoid) plus cycloheximide (50 mg dm^{-3}) to enumerate bacteria, *Pseudomonas* isolation agar (Oxoid), selective for pseudomonads and Czapek-Dox agar (Oxoid) plus ampicillin (10 mg dm^{-3}), streptomycin and tetracycline (50 mg dm^{-3}) for enumeration of fungi. Plates were incubated at 25 °C and colonies were counted weekly until no new growth was recorded.

2.4.2. Community level physiological profiles (CLPP)

Biolog® GN microplates (Biolog Inc., CA, USA), which contained 95 different carbon sources, were used together with exudate profile microplates, prepared using Biolog®-MT plates (Campbell et al., 1997), to construct a CLPP of the microbial community. A 50 cm^3 aliquot of the 10^{-4} dilution of the same soil samples used in the enumeration of culturable microorganisms was centrifuged at 2000 rpm for 10 min to separate soil and to minimise addition of soil or root derived carbon into the system. A 0.15 cm^3 aliquot of each sample was dispensed into each well of the GN and exudate plates, and the plates were incubated at 15 °C for 5 days. The colour development in the wells (carbon utilisation) was then measured as absorbance at 590 nm every 24 h using a microplate reader (Vmax, Molecular Devices, Oxford, UK).

2.5. Statistical analysis

2.5.1. Plant biomass data

In the statistical analysis of the shoot biomass data, the assessment of plant species effects, and

interactions involving species, is dependent both on the variability "between pots" and "within pots". Species effects are therefore assessed between pots for monoculture swards, and are assessed within pots for mixed swards. Consequently, a Restricted Maximum Likelihood (REML) analysis, allowing for blocking appropriate to the experimental design and allowing for both "between pot" and "within pot" variability, was used to assess the significance of the effects of larvae, species and sward plus all possible interactions, on the shoot biomass. Shoot data were log transformed prior to analysis. As the roots of the two species when grown as a mixture could not be separated, "within pot" variability was not an issue in the statistical analysis of the root data. Consequently, the root biomass and nutrient data were analysed using analysis of variance (ANOVA), with blocking appropriate to the experimental design. Contrasts were used to partition the treatment into effect of sward and effect of species. Both REML and ANOVA analyses were carried out using Genstat 5 for Windows (VSN International Ltd., Oxford, UK).

2.5.2. Root dynamics

For analysis of the root dynamics data, a hierarchical ANOVA including time as a factor, using Genstat 5 for Windows was adopted with no transformation required.

2.5.3. Microbial data

The microbial population data were log transformed prior to analysis of variance using the Genstat procedure ANOVA. For the CLPP data, the average well colour development (AWCD) of all 141 carbon (C) sources for each sample were calculated to eliminate variation in well colour development caused by different cell densities (Garland and Mills, 1991; Garland, 1996). The AWCD of different substrate groups (sugars, oligo-sugars, carboxylic acids, acidic amino acids, basic amino acids, neutral amino acids, amides, phenolic acids, alcohols, N–heterocyclic–N, long chain aliphatic acids) was calculated prior to performing the ANOVA. The CLPP data from each incubation time were also analysed using two forms of multivariate analysis, firstly by principal components analysis (PCA) to reduce the dimensionality in the data arising from having more variates than samples and then by canonical variate analysis (CVA) (Genstat 5.3). CVA differentiated samples based on their overall patterns of C utilisation and, by referring to ordination coordinate loadings, identified which C sources were more responsible for the discrimination. CVA of the root exudate C sources only was performed because this has been shown to improve the discriminating power of the technique, in addition to being more relevant to the rhizosphere (Campbell et al., 1997).

3. Results

3.1. Plant biomass

The introduction of larvae into the pots significantly reduced shoot biomass of *T. repens* both in monoculture and in the mixture, although the effect was greatest when in monoculture (Table 1). Conversely, the shoot biomass was not significantly reduced with larvae in *A. capillaris* (Table 1). Through daily visual inspection of the shoots, the effect of the larvae could be seen within a week of introduction, with the effect being most marked in the *T. repens* monoculture. Within the mixed species pots, the shoot biomass of *T. repens* was reduced when larvae were present, whereas there was no significant effect of larvae on the shoot biomass of *A. capillaris* (Table 1). The live root biomass of the plants grown as monocultures was reduced significantly by the presence of larvae ($P < 0.05$). The root biomass in the other pots was, however, unaffected by larvae (Table 1). Larval feeding changed the biomass partitioning in the *T. repens* monoculture pots, reducing the shoot:root ratio from 4.42 to 0.02 (without larvae and with larvae, respectively; $P < 0.001$). However, there was no significant change in the shoot:root ratio in the *A. capillaris* monoculture pots (1.56 without and 1.45 with larvae, respectively).

3.2. Root characteristics

The specific root length (SRL), i.e. length of root per unit root dry weight, was increased with larvae only in the *A. capillaris* monoculture pots (162 m g^{-1} with larvae and 110 m g^{-1} without, respectively; $P < 0.01$). There was no significant effect of larvae on SRL in the other treatments. The composition of the root system was altered by the presence of larvae. In the single species pots there were significantly greater

Table 1
Mean values (mg cm^{-2}, log transformed) for shoot dry weights with differences and standard error of differences, for *A. capillaris* and *T. repens* in mixture, and in monoculture, both with and without *T. paludosa* larvae

Plant part/species/sward	Without larvae	With larvae	Difference	S.E.D.
Shoots/*A. capillaris*/mixture	2.072	1.834	0.238	0.658
Shoots/*T. repens*/mixture	0.137	−1.389	1.526	0.658
Shoots/*A. capillaris*/monoculture	2.165	1.560	0.605	0.806
Shoots/*T. repens*/monoculture	1.434	−5.110	6.544	0.742
Roots/*A. capillaris* and *T. repens*/mixture	3.32	2.71	0.61	0.373
Roots/*A. capillaris*/monoculture	5.05	2.53	2.52	0.373
Roots/*T. repens*/monoculture	0.82	0.44	0.38	0.373

Effective degrees of freedom relating to shoots are 7 for between pots and 4 for within pots. Mean values (mg cm^{-2}, untransformed) for root dry weights with differences and standard error of differences, for *A. capillaris* and *T. repens* in mixture and in monoculture, both with and without *T. paludosa* larvae (degrees of freedom relating to roots are 7). χ^2-values for shoot data: species = 114.2***, larvae = 32.1***, species × larvae = 20.6***, species × sward × larvae = 14.0***, sward × larvae = 12.0***. *F*-values for root data: species = 77.9***, larvae = 29.3***, species × larvae = 9.9**. For the root data, "species" refers to species/sward combinations ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

($P < 0.01$) percentages of dead roots when the larvae were present (21% with larvae compared to 9% without larvae in *A. capillaris*, 27% with larvae in *T. repens* compared to 15% without).

3.3. Root nutrient content

There was no effect of larvae on the C concentration in the root tissue. However, there was a significant difference between species ($P < 0.001$) (Table 2). There was a significant larval × species interaction for root N concentration, with the larvae reducing N concentration in the root tissue of *T. repens* monocultures, but not that of *A. capillaris*. This was also reflected in an increase in the C:N ratio in *T. repens* monocultures, with larvae present.

3.4. Root dynamics

The rate of root production at the final harvest date was significantly reduced by the presence of the larvae in *A. capillaris* (Table 3). When the rate of root disappearance is examined over time for *T. repens* (Fig. 1), the effect of the larvae in increasing this rate was significant 2 days after the introduction of the larvae in *T. repens* only. In contrast, the rate of root disappearance only increased significantly 9 days after introduction of the larvae in the *A. capillaris* pots.

Table 2
Root tissue C and N concentration values (untransformed), $n = 3$, 10 d.f. (mean and LSD at $P \leq 0.05$), for *A. capillaris*, *T. repens* monoculture, and *A. capillaris* and *T. repens* mixture (species sward combination), both with and without *T. paludosa* larvae

	Without larvae			With larvae			LSD ($P < 0.05$ for larvae × species interaction)
	Monoculture		Mixture	Monoculture		Mixture	
	A. capillaris	*T. repens*	*A. capillaris*/*T. repens*	*A. capillaris*	*T. repens*	*A. capillaris*/*T. repens*	
C concentration (mg C g^{-1} root)	437 a	480 b	450 a	448 a	473 b	441 a	15.0
N concentration (mg N g^{-1} root)	14.9 ab	28.2 d	15.6 bc	13.1 a	17.2 c	14.6 ab	2.37
C:N ratio	29.5 b	17.0 a	28.8 b	34.3 c	27.7 b	30.3 bc	4.42

Contrasting letters within a row denote a statistically significant difference ($P < 0.05$). *F*-values for % C: species = 28.22***; for % N: species = 79.64***, larvae = 55.77***, species × larvae = 27.01***; for C:N species = 25.02***, larvae = 24.63***, species × larvae = 5.54*. Species refers to species/sward combinations ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

Table 3
Daily rates of root appearance (number cm^{-3} day^{-1}) at harvest (untransformed) for *A. capillaris*, *T. repens* monocultures and *A. capillaris* and *T. repens* mixture (species sward combination), both with and without *T. paludosa* larvae

	Without larvae			With larvae			LSD ($P < 0.05$ for larvae × species interaction)
	Monoculture		Mixture	Monoculture		Mixture	
	A. capillaris	*T. repens*	*A. capillaris*/*T. repens*	*A. capillaris*	*T. repens*	*A. capillaris*/*T. repens*	
Root appearance rate	8.04 b	0.68 a	4.99 ab	2.18 a	0.95 a	3.13 a	4.59

Values calculated on $n = 3$, 10 d.f. and from a volume of 0.8 cm^3 soil from each minirhizotron. Contrasting letters within a row denote a statistically significant difference ($P < 0.05$). *F*-values for larvae = 4.6*, for species = 4.7*. Species refers to species/sward combinations (* $P < 0.05$).

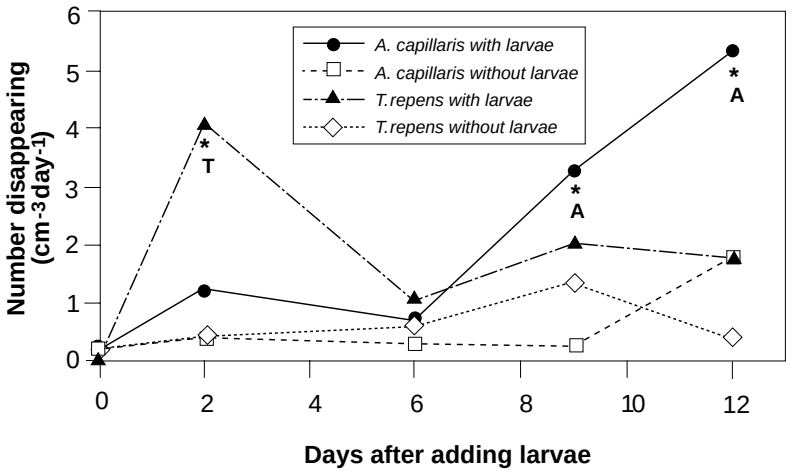

Fig. 1. Effect of *T. paludosa* larvae, on the rate of root disappearance from the soil (number cm^{-3} day^{-1}) from a volume of 0.8 cm^3 on for each minirhizotron. $*_T$ represents a statistically significant difference between *T. repens* pots and $*_A$ represents a statistically significant difference at that date between *A. capillaris* pots, with and without larvae treatment, $P < 0.05$, $n = 3$. *F*-values for species = 14.52**, time = 4.44*, time × species = 3.35* (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Larvae were added to the pots on day 0.

Table 4
Number (log cfu g^{-1} soil) of total culturable bacteria and pseudomonads in the rhizosphere of *T. repens*, *A. capillaris* and a mixture of *T. repens* and *A. capillaris*, all with, and without *T. paludosa* larvae

	T. repens (−larvae)	*T. repens* (+larvae)	*A. capillaris* (−larvae)	*A. capillaris* (+larvae)	*T. repens*/*A. capillaris* mixture (−larvae)	*T. repens*/*A. capillaris* mixture (+larvae)	LSD ($P < 0.005$ larvae × species interaction)
Total bacteria (log cfu g^{-1} soil)	17.39 a	16.98 a	16.98 a	17.35 a	16.57 a	16.94 a	0.998
Pseudomonads (log cfu g^{-1} soil)	11.66 a	14.32 b	11.74 a	14.39 b	11.47 a	14.49 b	1.571

Values are the mean of three with 10 d.f. and values a and b denote a significant difference at $P < 0.05$. Contrasting letters within a row denote a statistically significant difference ($P < 0.05$). For pseudomonads, *F* for larvae = 5.77*, for species × larvae = 19.35***. Species refers to species/sward combinations (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

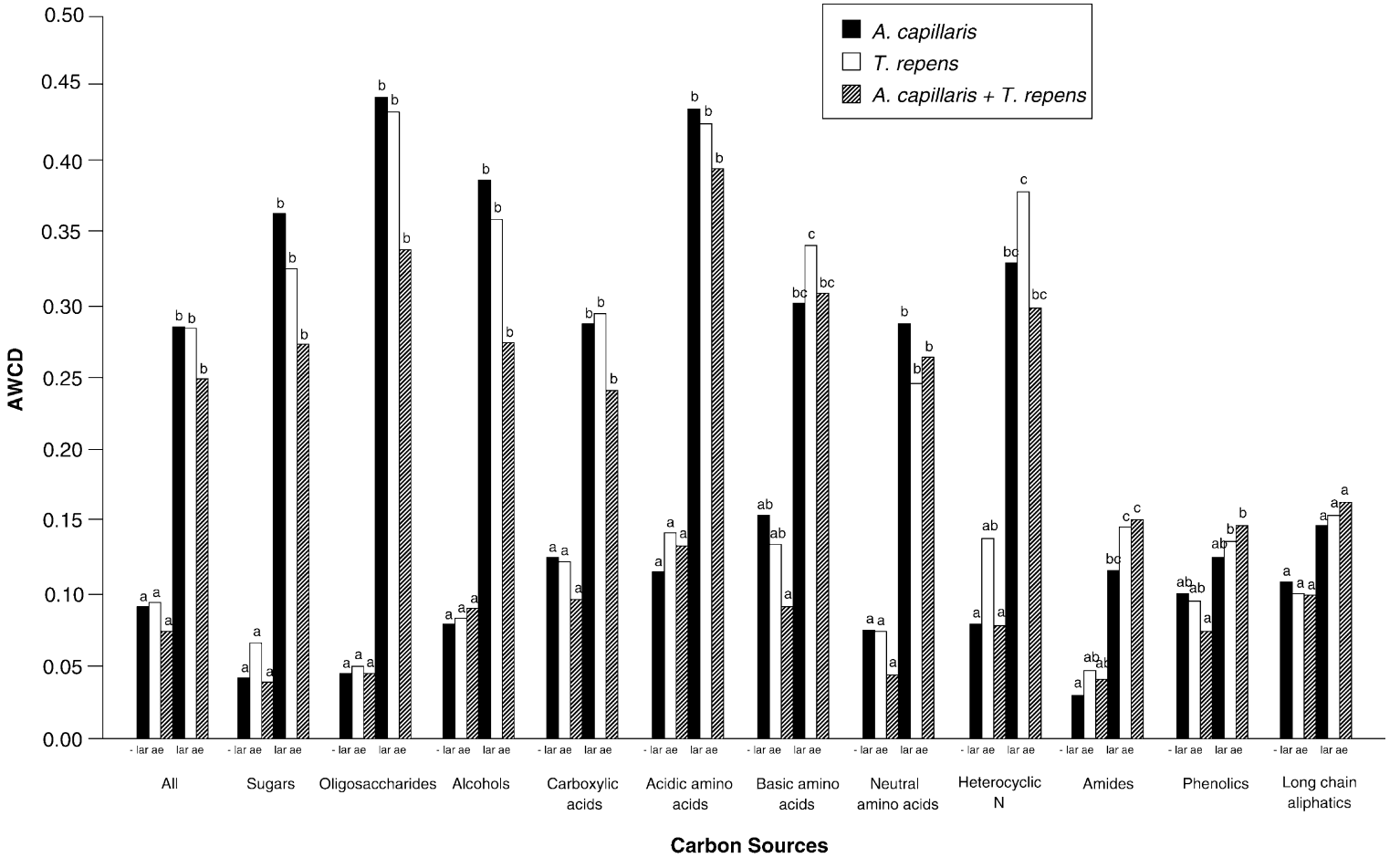

Fig. 2. The average well colour development (AWCD), for the main groups of carbon compounds in the Biolog GN and exudate plates by microbial communities from the rhizosphere of *T. repens*, *A. capillaris*, and a mixture of *T. repens* and *A. capillaris* in the presence and absence of *T. paludosa* larvae. (Untransformed means are presented and a, b, c values denote a statistically significant difference within each substrate on log transformed data at $P < 0.05$, $n = 3$). $LSD_{0.05}$, for C sources are all 0.148, sugars 0.177, oligosaccharides 0.237, alcohols 0.208, carboxylic acids 0.151, acidic amino acids 0.252, basic amino acids 0.195, neutral amino acids 0.159, heterocyclic N 0.214, amides 0.089, phenolics 0.055, long chain aliphatics 0.064.

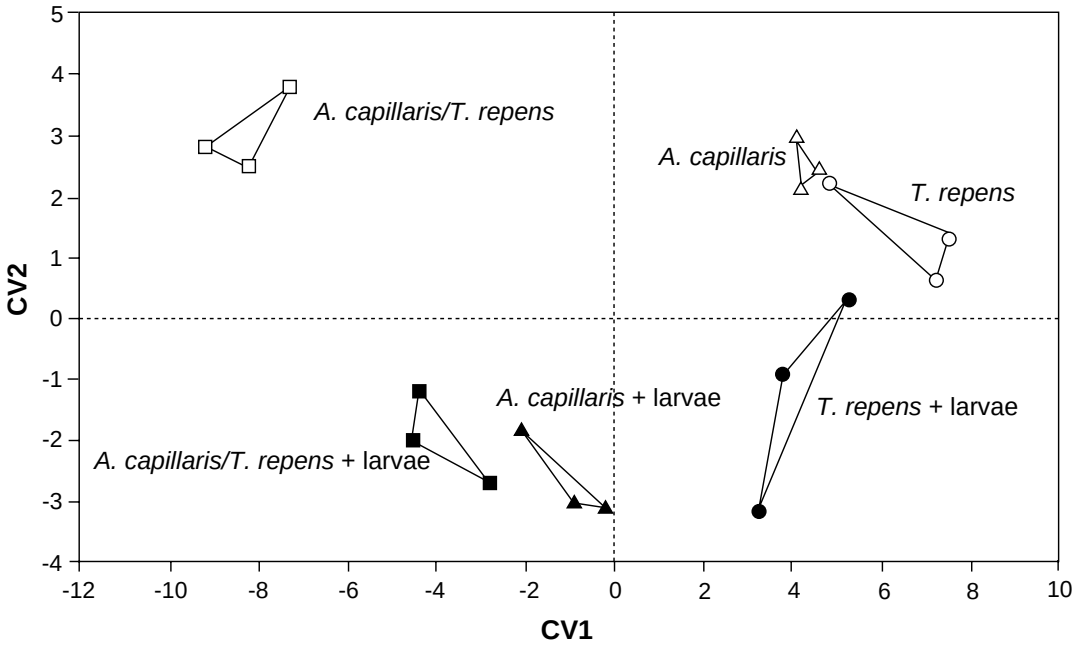

Fig. 3. Canonical variate analysis of soil samples based on CLPP of microbial communities from the rhizosphere of *T. repens*, *A. capillaris* and a mixture of *T. repens* and *A. capillaris*, in the presence and absence of *T. paludosa* larvae. (Values are for 72 h incubation).

3.5. Rhizosphere microbial community

The total number of bacteria cultured on plates was not affected significantly by the presence of larvae in any of the treatments (Table 4). Numbers of culturable pseudomonads were increased significantly in all sward types in the presence of larvae (Table 4). Populations of yeasts and fungi were not affected significantly by any treatment (data not shown). Analysis of the CLPP of the microbial communities showed that the microorganisms in the soil from pots containing larvae had significantly greater and faster utilisation of all groups of carbon compounds, with the exception of phenolic and long chain aliphatic acids, than those from the same type of sward without larvae (Fig. 2). CVA differentiated the samples from pots with and without larvae on canonical variate axis 2 (CV2) (Fig. 3). Further analysis of the loadings of the carbon sources on this CV axis indicated that some sugars (arabinose, galactose, maltose), carboxylic (tartaric, malic, oxalic) and amino acids (lysine, serine) were mainly responsible for the discrimination between presence and absence of larvae. The samples also separated out on CV1, particularly for *T. repens* which had high values on CV1, due to greater utilisation of quercetin, hesperetin, oxalic acid and L-tyrosine by microbial communities in pots containing *T. repens*.

4. Discussion

4.1. Larval influence on plant species

The presence of tipulid larvae significantly reduced the shoot biomass of *T. repens* when grown in monoculture and when present in the mixture, in contrast to *A. capillaris*, where there was no significant effect measured. The effects were also most visible on the *T. repens* shoots, with direct feeding observed. These results suggest a feeding preference of tipulids for *T. repens*. Ramsell et al. (1993) showed that *T. paludosa* larvae demonstrated preference for *L. perenne* over *Rumex obtusifolius*. In a field study, White and French (1968) showed that grazing by *Tipula* spp. reduced the biomass of *Trifolium* spp. to a greater extent than grasses when in a mixed sward. The grass

grub (*Costelytra zealandica*) has also been shown to prefer *Trifolium* spp. to other pasture species (Gaynor et al., 1986; Prestidge et al., 1985). This has been attributed to low levels of chemical deterrent in *Trifolium* (Gaynor et al., 1986). However, white clover has the potential to produce HCN when damaged (Daday, 1985) which is known to deter feeding by slugs and has been shown to reduce damage by *Sitona* spp. (Mowatt and Shakel, 1989). However, Murray (1996) found that feeding by *Sitona* spp. on lines of white clover with high HCN potential was greater than on lines with lower HCN potential.

In the monoculture swards, the root biomass of both species was significantly reduced by larval herbivory. In *A. capillaris*, this corresponded to a significant reduction in the rate of new root production and a significant increase in the rate of root disappearance at the time of harvest, as a result of larval herbivory. The specific root length increased with the addition of larvae in *A. capillaris*, possibly reflecting a reduction in overall diameter due to removal of older root axes and replacement by newer and thinner roots. However, the larvae significantly increased the rate of root disappearance in *T. repens* sooner than in *A. capillaris*, again suggesting a different mode of herbivory between the two species. In addition, the tipulid grazing reduced root N concentration in *T. repens* only. Direct leakage of N from damaged roots has been considered to be one mechanism for this reduction, and has been observed in *T. repens* roots damaged by *Sitona* spp. larvae (Murray and Clements, 1999). Larvae may penetrate the xylem (Powell and Campbell, 1983), which could lead to leakage of N containing compounds. The significantly greater N concentration in *T. repens* roots compared to *A. capillaris* roots may have allowed the larvae to remove a smaller amount of root tissue in absolute biomass terms from *T. repens* than from *A. capillaris* in order to satisfy their growth requirements. In addition, the larvae preferentially damaged and removed the leaf material in *T. repens* leading to a significant reduction in the shoot:root ratio in the *T. repens* monoculture pots. Ramsell et al. (1993) showed that *T. paludosa*, significantly reduced the root biomass of *L. perenne* in both monoculture and in mixtures but the grazed *L. perenne* was found to subsequently be a stronger competitor compared to *R. obtusifolius*, due to enhanced shoot growth rates. *A. capillaris*, with its younger root system after herbivory, may also become a better competitor against *T. repens*, when subject to root herbivory. Working with *S. hispidulus*, Murray and Hatch (1994) found extensive damage to clover root nodules, root hairs and rootlets, but no damage was observed on roots of *L. perenne*. In addition, in a root feeding study of larvae of *T. paludosa*, a reduction in root length of 15% in 7 days was observed in *T. repens*, whereas there was no effect on root length in *L. perenne* (Dawson et al., 2002). These studies suggest that feeding preferences by insect larvae can be very species specific, and that differential shoot and root grazing in mixtures can have important consequences for plant competition and community composition in grasslands.

4.2. Effects on microbial community

The soil microbial community structure was altered in the presence of larvae, with a significantly higher population of pseudomonads being present in pots with *T. paludosa*. In addition, there was a change in the metabolic profile of the microbial community when larvae were present, in all sward types studied. Denton et al. (1999) found that root herbivory by clover cyst nematodes significantly increased the soil microbial biomass, and they hypothesised that nematode herbivory increased root exudation, as had been shown by Yeates et al. (1998). The increase in *Pseudomonas* spp. abundance in this study reflects their fast growth rate in soil, and there is increasing evidence that they can be selectively stimulated in the rhizosphere of a range of plant species (Grayston et al., 1998; Marilley and Aragno, 1999; McCaig et al., 2001). Murray et al. (1996) showed changes in root exudation patterns from white clover as a result of *Sitona* spp. larval feeding, particularly changes in the amino acid profiles. In addition, the larvae increased the proportion of necromass present in the soil. This increase in dead root material from an increased root disappearance, whether directly by root herbivory or indirectly by shoot herbivory and death, can act as a resource for decomposer organisms. In addition, there would be an increase in amounts of faecal material in the soil, which have the potential to be microsites of microbial activity. The presence of the larvae led to a change in the CLPP of the soil microbial community which was mainly due to differences in sugars, carboxylic

and amino acid usage, suggesting larval herbivory increases the release of these compounds, which then selects for microorganisms capable of utilising these substrates. The CLPP analysis also discriminated between microbial communities from pots containing *T. repens* and the other sward types due to greater utilisation of a number of phenolic acid signal molecules by the former communities. In particular, quercetin and hesperetin have been identified as host-specific recognition signals produced by legumes to stimulate arbuscular mycorrhizal symbiosis, as reviewed by Grayston et al. (1996). Shoot herbivory by *T. paludosa* may also have played a part in altering the microbial community, in that partial shoot defoliation has been found to result in increased release of organic compounds from roots, in particular, low molecular weight soluble exudates (Holland et al., 1996). Paterson and Sim (1999, 2000) found transient increases in release of organic compounds from roots of *L. perenne* and *F. rubra* following shoot defoliation. In addition, shoot defoliation has also been shown to alter the soil microbial community (Dawson et al., 2000).

5. Conclusions

Herbivory by *T. paludosa* larvae, whether through shoot or root herbivory, can rapidly impact upon plant growth and also upon the structure and function of the microbial community. In addition, the effects can be species specific. Future studies will help elucidate the interactions between herbivory and the stimulation and characterisation of root exudation, root death, larval defecation, decomposition and the soil microbiota. Whether the mechanism of the dramatic increase in pseudomonad populations in the presence of larvae is increased root exudation, root damage and leakage, root death, larval defecation or activity is still to be determined. The results observed in this study will impact on the understanding of vegetation dynamics and have consequences for pasture management.

Acknowledgements

This work was funded by the Scottish Executive Rural Affairs Department and NERC (as part of the Soil Biodiversity Thematic programme). IGER is supported by the BBSRC. The authors would like to thank Dr. Peter Dennis (Macaulay) for assistance in identifying the larvae and Elizabeth Duff (BioSS) for advice on the statistical analysis of plant data.

References

Blackshaw, R.P., 1984. The impact of low numbers of leather jackets on grass yield. Grass Forage Sci. 39, 339–344.

Blackshaw, R.P., Newbold, J.W., 1987. Interactions of fertilizer use and leather jacket control in grassland. Grass Forage Sci. 42, 342–348.

Brown, V.K., Gange, A.C., 1989. Differential effects of above- and below-ground insect herbivory during early plant succession. Oikos 54, 67–76.

Brown, V.K., Gange, A.C., 1991. Root herbivory and vegetation dynamics. In: Atkinson, D. (Ed.), Plant Root Growth: An Ecological Perspective. Blackwell Scientific Publications, Oxford, p. 478.

Campbell, C.D., Grayston, S.J., Hirst, D.J., 1997. Use of rhizosphere carbon sources in sole carbon utilization tests to discriminate soil microbial communities. J. Microbiol. Meth. 30, 33–41.

Clements, R.O., 1984. Control of insect pests in grassland. Span 27, 77–80.

Clements, R.O., Murray, P.J., Bentley, E.R., Lewis, G.C., French, N., 1990. The impact of pests and diseases on the herbage yield of permanent grassland at eight sites in England and Wales. Ann. Appl. Biol. 117, 349–357.

Coulson, J.C., Butterfield, J., 1978. Animal communities of upland Britain. The Future of Upland Britain. Centre for Agricultural Research, Reading University. Bulletin No. 2, pp. 417–436.

Daday, H., 1985. Cynogenesis in strains of white clover (*Trifolium repens* L.). J. Br. Grassland Soc. 10, 266–279.

Dawson, L.A., Grayston, S.J., Paterson, E., 2000. Effects of grazing on the roots and rhizosphere of grasses. In: Lemaire, G., Hodgson, J., de Moraes, A., Carvalho, P.C., Nabinger, C. (Eds.), Grassland Ecophysiology and Grazing Ecology. CABI Publishing, Cambridge, Chapter 4, pp. 61–84.

Dawson, L.A., Grayston, S.J., Murray, P.J., Pratt, S.M., 2002. Root feeding behaviour of *Tipula paludosa* (Meig.) (Diptrea:Tipulidae) on *Lolium perenne* (L.) and *Trifolium repens* (L.). Soil Biol. Biochem. 34, 609–615.

Denton, C.S., Bardgett, R.D., Cool, R., Hobbs, P.J., 1999. Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. Soil Biol. Biochem. 31, 155–165.

French, N., 1969. Assessment of leather jacket damage to grassland and economic aspects of control. Proc. 5th Br. Insecticide Fungicide Conf. 2, 511–521.

French, N., Nichols, D.B.R., Wright, A.J., 1990. Yield response of improved upland pasture to the control of leather jackets under increasing rates of nitrogen. Grass Forage Sci. 45, 99–102.

Garland, J.L., 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biol. Biochem. 28, 213–221.

Garland, J.L., Mills, A.L., 1991. Classification and characterisation of heterotrophic microbial communities on the basis of patterns of community-level-sole-carbon-source utilisation. Appl. Environ. Microb. 57, 2351–2359.

Gaynor, D.L., Lane, G.A., Biggs, D.R., Sutherland, O.R.W., 1986. Measurement of grass grub resistance of bean in a controlled environment. N. Z. J. Exp. Agric. 14, 77–82.

Grayston, S.J., Vauhan, D., Jones, D., 1996. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl. Soil Ecol. 5, 29–56.

Grayston, S.J., Wang, S., Campbell, C.D., Edwards, A.C., 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem. 30, 369–378.

Holland, J.N., Cheng, W., Crossley Jr., D.A., 1996. Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. Oecologia 107, 87–94.

Marilley, L., Aragno, M., 1999. Phylogenetic diversity of bacterial communities differing in degree of proximity to *Lolium perenne* and *Trifolium repens* roots. Appl. Soil Ecol. 13, 127–136.

McCaig, A.E., Grayston, S.J., Prosser, J.I., Glover, L.A., 2001. Impact of cultivation on characterisation of species composition of soil bacterial communities. FEMS Microbiol. Ecol. 35, 37–48.

Mowatt, D.J., Jess, S., 1986. The control of leather jackets *Tipula paludosa* Meig., in grassland by early application of insecticide. Grass Forage Sci. 41, 27–30.

Mowatt, D.J., Shakel, M.A., 1989. The effect of different cultivars of clover on numbers of, and leaf damage by, some invertebrate species. Grass Forage Sci. 44, 11–18.

Murray, P.J., 1996. Evaluation of a range of varieties of white clover for resistance to feeding by weevils of the genus *Sitona*. Plant Var. Seeds 9, 9–14.

Murray, P.J., Clements, R.O., 1992. Studies on the feeding of *Sitona lineatus* L. (Coleoptera:Curculionidae) on white clover (*Trifolium repens* L.) seedlings. Ann. Appl. Biol. 121, 233–238.

Murray, P.J., Hatch, D.J., 1994. *Sitona* weevils (Coleoptera: Curculionidae) as agents for rapid transfer of nitrogen from white clover (*Trifolium repens* L.) to perennial ryegrass (*Lolium perenne* L.). Ann. Appl. Biol. 125, 29–33.

Murray, P.J., Clements, R.O., 1999. *Sitona* spp. (Coleoptera: Curculionidae) in grassland in England and the impact of their root herbivory on white clover. Antenna 23, 221–227.

Murray, P.J., Hatch, D.T., Cliquet, J.B., 1996. Impact of insect root herbivory on the growth and nitrogen and carbon contents of white clover (*Trifolium repens*) seedlings. Can. J. Bot. 74, 1591–1595.

Murray, P.J., Dawson, L.A., Grayston, S.J., 2002. Influence of root herbivory on growth response and carbon assimilation by white clover plants. Appl. Soil Ecol. 20, 97–105.

Newbold, J.W., 1981. The control of leather jackets in grassland by winter pesticide applications. Proc. Crop Protection in Northern Britain, pp. 207–211.

Newman, E.I., 1996. A method of estimating the total length of root in a sample. J. Appl. Ecol. 3, 139–145.

Paterson, E., Sim, A., 1999. Rhizodeposition and C-partitioning of *Lolium perenne* in axenic culture affected by nitrogen supply and defoliation. Plant Soil 216, 155–164.

Paterson, E., Sim, A., 2000. Effect of nitrogen supply and defoliation on loss of organic compounds from roots of *Festuca rubra*. J. Exp. Bot. 51, 1449–1457.

Powell, G.S., Campbell, W.V., 1983. Histological examination of larval clover root curculio (Coleoptera:Curculionidae) damage to ladino white clover. J. Econ. Entomol. 76, 741–743.

Prestidge, R.A., van der Zipp, S., Badan, D., 1985. Effects of plant species and fertilizers on grass grub larvae *Costelytra zealandica*. N. Z. J. Agric. Res. 28, 409–417.

Ramsell, J., Malloch, A.J.C., Whittaker, J.B., 1993. When grazed by *Tipula paludosa*, *Lolium perenne* is a stronger competitor of *Rumex obtusifolius*. J. Ecol. 81, 777–786.

Rodwell, J.S., 1992. British Plant Communities, vol. 3. Cambridge University Press, Cambridge, p. 540.

Tennant, D., 1975. A test of a modified line intersect method of estimating root length. J. Ecol. 63, 995–1001.

White, J.H., French, N., 1968. Leather jacket damage to grassland. J. Br. Grassland Soc. 23, 326–329.

Yeates, G.W., Saggar, S., Denton, C.S., Mercer, C.F., 1998. Impact of white clover cyst nematode (*Heterodera trifolii*) infection on soil microbial activity in the rhizosphere of white clover (*Trifolium repens*): a pulse labelling experiment. Nematologica 744, 81–90.