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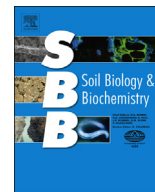
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# Assessing the impact of agricultural forage crops on soil biodiversity and abundance



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## ABSTRACT

Maintaining soil biodiversity and function is key to maintaining soil health, nutrient cycling and decomposition. Different forage species have variable concentrations of essential nutrients and rooting patterns, potentially affecting soil biology and soil–plant–animal interactions. Our study compared the effect of growing different forage crops on soil faunal diversity and abundance. Plots of chicory (*Cichorium intybus*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*) or perennial ryegrass (*Lolium perenne*) were established in 2009 and maintained over a four year period. Soil faunal samples were taken, including soil mesofauna, nematodes and earthworms, at the end of this period in autumn 2012 and spring 2013. Significant differences were found between the forages for a number of biological groups, as well as some seasonal differences; overall earthworm abundance and biomass was higher within the white clover treatment, specifically anecic earthworms. Nematode functional groups were found to differ, with greater numbers of fungal feeders in the clovers and chicory treatments, whilst the herbivores had the greatest abundances in the two ryegrass treatments. Overall the microarthropod order abundances did not differ, however two collembolan superfamilies did show differences between treatments with the detritivorous Poduromorpha having a higher abundance in the clovers and chicory treatment and the herbivorous Symphypleona had a higher abundance in the ryegrass treatment. Relatively little is known about the links between soil biology and the effects of plant type because of the complex nature of soil, however here we have begun to reveal some of these linkages. Overall, the findings indicate a relationship between ryegrass and herbivorous invertebrates, whilst the other forages have a stronger relationship with decomposer invertebrates; changing the dominance within the soil food web dependent on forage type.

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## 1. Introduction

There is increasing pressure from society for farming systems to deliver “ecosystem services” as well as feeding the world, and soil biology are vital in providing these services (Ferris and Tuomisto, 2015). The main ecosystem services a farming system provides are “provisioning” (food production), however other provided services that are considered include “regulating”, “cultural” or “supporting” (Millennium Ecosystem Assessment, 2005). Agricultural production changes the abundance and diversity of soil biology, affecting the ecosystem services in both the short and long

term. Soil fauna as a complete food web are important in the maintenance of plant production (Hunt and Wall, 2002). Maintaining a healthy soil will allow the soil biota to support nutrient cycling, decomposition and regulate the environment. Promoting the maintenance of a “healthy” soil could increase yields in the long term, with direct feedbacks between the above-ground and below-ground ecosystems (Wardle et al., 2004). Soil fauna could be a utilisable tool in sustainable agriculture (Paoletti, 1999), assessing abundance and diversity could be a proxy to monitor soil health, as there is often less diversity within agricultural soil due to management practices (Firbank et al., 2008). The soil fauna have a large amount of functional redundancy; however changes to soil health will occur if particularly influential species are lost (Nielsen et al., 2011).

Agricultural profitability and sustainability are crucial to farming successfully. Incorporating legumes into agricultural systems is thought to save farmers 137 € ha<sup>-1</sup> across Europe through

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reduction in inorganic N use (Rochon et al., 2004). Forage legumes and chicory (*Cichorium intybus*) differ in their micronutrients concentrations (Marley et al., 2013a,b), increase the seasonal availability of high quality forage (Hare et al., 1987); and improve feed intake in livestock in comparison to feeding ryegrass (*Lolium perenne*) forage (Marley et al., 2007). The ability of legumes to fix atmospheric N has been demonstrated in numerous studies globally (Bullied et al., 2002; Evans et al., 2003; Marley et al., 2013c) and have also been found to have quantitatively more stable associations with mycorrhizal fungi than ryegrass (Zhu et al., 2000). Both red (*Trifolium pratense*) and white clover (*Trifolium repens*) have started to be used as a cover crop or living green manure as part of a crop rotation, reducing the risk of soil erosion and providing a residual N source for companion and future crops (Rasmussen et al., 2012) or soil fauna through root exudation. To enable the development of sustainable agriculture, there is a need now to understand how all crops affect belowground food webs.

Most of the biodiversity within agricultural systems resides in the soil (Brussaard et al., 2007), exceeding above-ground diversity by several orders of magnitude (Anderson, 2009). Agricultural grasslands support a relatively stable and numerous soil biota that contribute to soil functioning and fertility (Murray et al., 2012). Earthworms are ecosystem engineers (Jones et al., 1994), because of their large effect on the soil environment (Blouin et al., 2013). Microarthropods also have significant effects on belowground processes, contributing to the carbon/nitrogen cycles and litter decomposition (Osler and Sommerkorn, 2007) as well as improving soil aggregation (Siddiky et al., 2012), dispersing fungal spores (Dromph, 2001) and even affecting plant succession (Bonkowski and Roy, 2012). Maintaining an optimal environment suitable for these ecosystem engineers and key participants in nutrient cycling, should provide a cascade in benefits through the different functional groups within the soil and is key to good soil management and maintaining a healthy soil food web.

Variability among rooting systems and sward cover of plant species, leads to differences in their effects on overall productivity, stability of soil, microbial processes (White et al., 2013) and the soil food web itself. For example, perennial ryegrass has a shallow but extensive rooting system that is highly branched and produces fine adventitious roots, whereas chicory and (to a lesser extent) red clover produce deep tap roots that have the potential to 'mine' deep soil resources that are inaccessible to other shallower rooting plants (Belesky et al., 2001). Whilst white clover after initially producing a short tap root spreads through the production of stolons at root nodules (Marriott and Haystead, 1992). Pores created by chicory's taproot, have been found to provide better access for the next crop to the nutrients located within deeper soil (Perkons et al., 2014), increased porosity has also been found to increase earthworm abundance (Kautz et al., 2014). These differences in root architecture affect the soil ecosystem, changing the soil faunal assemblage to those best adapted to the environment they are residing in (Bonkowski, 2004).

Studies have focused on whether the soil food web is driven from litter or root inputs, utilising stable isotopes (Pollierer et al., 2007), or whether the soil food web drives plant species diversity (Bennett, 2010), however few look at the effects of specific plant species on the food web itself. To further our understanding of the effect of different forages on soil fauna, an experiment was set up to test the hypothesis that alternative forages to ryegrass, such as forbs/legumes, would alter the soil habitat. The hypothesis we investigated, was that a change in soil habitat (plant type) will influence the environment and should be reflected in the faunal biodiversity residing there; these differences will potentially be linked to the specific attributes of the different forages, e.g. tap roots, nitrogen fixation, or mycorrhizal association, and be visible

through differences in abundance and diversity of the soil fauna. Specifically we hypothesise that the differences in forage rooting system (fine/extensive for ryegrass, tap roots/stolons for chicory and clovers) will favour different functional groups of soil fauna to a lesser or greater extent. Also those forages that are more strongly mycorrhizal will favour soil faunal functional groups linked to fungivory.

## 2. Material and methods

### 2.1. Experimental site, plot characteristics and maintenance

Twenty plots (7.5 m × 12 m) were set up at the Institute of Biological, Environmental and Rural Sciences (IBERS), University of Aberystwyth, Wales (52° 25' 59" N, 4° 1' 26" W) in June 2009. Plots were set up on an area of stony, well-drained loam of the Rheidol soil series (see Table 1 for site characteristics). Soil temperatures are typical of a temperate European climate in this area of the UK with the 50 year average ranging from 3.8 °C in winter to 16.8 °C in summer. The experimental area was ploughed to a uniform depth and soil nutrient status was standardised, the area received ground dolomitic limestone (magnesium lime) at 5 t ha<sup>-1</sup> to achieve a soil pH of 6.0–6.5 (pre-cultivation pH = 5.75; Mg = 65.5 mg L<sup>-1</sup>; Ca 1279.5 mg L<sup>-1</sup>). Soil P and K were amended using a muriate of potash applied at the rate of 60 kg K<sub>2</sub>CO<sub>3</sub> and triple super phosphate at the rate of 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (pre-cultivation P = 33.5 mg L<sup>-1</sup> and K = 125.1 mg L<sup>-1</sup>). Initial soil analysis was performed prior to setting up the experimental plots as in Crotty et al. (2014); mineral soil analysis (Table 1) (0–7.5 cm cores) was determined for ammonium-N (NH<sub>4</sub>-N), nitrate (NO<sub>3</sub>-N), phosphorus, potassium, calcium, and magnesium. Soil minerals were extracted using acetic acid and measured by inductively coupled plasma optical emission spectroscopy (ICP-OES). Soil pH was determined as 1:1 (soil:water) mixture, shaken for 30 min prior to pH measure. Field plots of the five treatments were established in a randomised block design (n = 4). Perennial ryegrass (*L. perenne*) (cv. Premium) with minimal input of inorganic N ha<sup>-1</sup> (80 kg N ha<sup>-1</sup> applied during the three years prior to the experiment) (Low N), perennial ryegrass plus 200 kg N ha<sup>-1</sup> annum<sup>-1</sup> (200 N), chicory (*C. intybus*; cv. Puna II) plus 200 kg N ha<sup>-1</sup> annum<sup>-1</sup>, white clover (*T. repens*; cv. AberDai) and red clover (*T. pratense*; cv. Merviot) were established using seed rates of 33, 33, 6, 6 and 16 kg ha<sup>-1</sup>, respectively.

During 2010–2012, fertiliser was applied as ammonium nitrate (Yara Ltd, Grimsby, UK) mid-March, and then immediately after the 1st, 2nd and 3rd harvesting cuts at a rate of 80, 60, 30 and 30 kg N,

**Table 1**

Site characteristics, previous cropping, soil analysis and meteorological data (mean ± standard error). Met Office weather station data located at Gogerddan.

<i>Location characteristics</i>	
UK Ordinance Survey Grid ref	52° 25' 59" N, 4° 1' 26" W
Altitude (a.s.l.)	30 m
Soil series	Rheidol
Soil type	Stony, loam
Drainage status	Well-drained
Site history	Grass
Soil temperature (at 10 cm) (°C) 50 year average	9.7 (±1.41)
<i>Soil analysis (autumn 2012)</i>	
pH (H <sub>2</sub> O)	6.2 (±0.03)
Ammonium-N (mg kg <sup>-1</sup> DM)	6.25 (±0.374)
Nitrate N (mg kg <sup>-1</sup> DM)	7.15 (±0.235)
Organic C (g/kg <sup>-1</sup> )	29.26 (±0.581)
Phosphorus (ppm)	22.0 (±0.64)
Potassium (mg kg <sup>-1</sup> )	90.6 (±4.25)
Calcium (mg kg <sup>-1</sup> )	1064.4 (±27.99)
Magnesium (mg kg <sup>-1</sup> )	189.2 (±4.46)

respectively. Nitrogen fertiliser was not applied to the red clover or white clover plots. Soil P and K were maintained by applying a muriate of potash and triple super phosphate annually as described above. Herbage cover was sprayed out between plots to avoid clover introgression between treatments. Red and white clover plots were treated with a herbicide (Kerb; Dow Agro Sciences, Hitchin, UK) in February 2010 and February 2011. All plots were mechanically harvested at the same time at five week intervals during the growing season annually to simulate silage cutting. All forages were cut at a height of 8 cm using a Haldrup 1500 plot harvester (J. Haldrup a/s, Løgstør, Denmark) and the material removed. The dry matter yields of the experimental plots were deemed as representative for each forage type (Marley et al., 2013b; Rhymes et al., 2015).

## 2.2. Soil fauna sampling

In September 2012 and March 2013, the abundance of key functional groups in the soil food web, including earthworms, nematodes and microarthropods were quantified. Soil was taken from randomised areas within each plot for each of the faunal groups. The areas where earthworm samples were taken was randomised and recorded so that the same area was not resampled the following spring due to the size of the sample compared to the other faunal measures.

### 2.2.1. Earthworm population assessment

Earthworm biomass ( $\text{g m}^{-2}$ ), abundance ( $\text{m}^{-2}$ ) and diversity were quantified from a cube of soil  $30 \text{ cm}^{-2}$  excavated to a depth of 30 cm from each plot and hand-sorted. After excavation, 0.5% formaldehyde solution was added to the pit to expel deep-burrowing earthworms; these were removed and added to the respective block sample. During hand-sorting, all resident earthworms were removed, maturity level assessed and grouped into adult (clitellate) and juvenile species prior to counting and weighing. Mature species were stored in alcohol and identified to species (Sherlock, 2012) to confirm live identifications.

### 2.2.2. Nematode population assessment

Twenty soil samples were collected to a depth of 15 cm from across each plot using a 4 cm diameter auger. A 200 g soil sample was taken to assess dry weight by oven drying at  $105^\circ\text{C}$  for 24 h. Whilst 200 g of fresh soil was used for nematode wet tray extractions based on the methods of Whitehead and Hemming (1965) and adapted by Crotty et al. (2011). Using the soil dry weight the total number of nematodes per gram dry soil for each plot was calculated. Samples were reduced through siphoning, to 5 ml, transferred to sample tubes with an equal volume of 8% formaldehyde (Merck, Poole, UK) added and the sample stored at  $4^\circ\text{C}$  until identifications of nematode functional groups were performed. Nematode feeding functional groups were identified using Adl (2003); adapting the method of Freckman and Ettema (1993), 100 nematodes per stored sample were randomly selected and identified to functional group, using an inverted compound microscope.

### 2.2.3. Microarthropod sampling

Microarthropods were sampled from three intact soil cores (5 cm diameter, 10 cm depth) collected from each replicate plot and placed together upside down in Tullgren funnels for extraction over seven days. Invertebrates migrate through each core via a temperature gradient (Crotty et al., 2012) and were extracted into 70% alcohol, prior to being counted and identified using a microscope, separating into the main superfamilies/lineages for Collembola and mites (Hopkin, 2007; Krantz and Walter, 2009) as well as

identifying the other invertebrates to order (Tilling, 1987). The Simpson index of diversity (1-D), a measure of community composition of all microarthropods extracted (Collembola and mites main superfamilies/lineages, Coleoptera larvae, Diptera larvae, Enchytraeidae, earthworms, spiders, Hemiptera, Coleoptera, Chilopoda, Diplopoda, Diptera, Pauropoda, Protura, Symphyla and Thysanoptera), was calculated from the equation:

$$1 - \sum_{i=1}^s n_i(n_i - 1) / N(N - 1)$$

where  $n_i$  is the number of organisms of species  $i$  and  $N$  the total number of organisms of all  $s$  species within each habitat.

### 2.2.4. Statistical analysis

All data were analysed with the aid of GenStat® (Payne et al., 2014). Data collected from the randomised block design over two sampling times were analysed assuming a split plot design, with effects of forage treatment estimated in the whole plot stratum and effects of sampling time and forage–time interactions estimated at the sub plot level. Where necessary data were normalised prior to univariate and multivariate analysis of variance. Where applicable, multiple comparisons were made using the Student Newman Keuls test (SNK) or in one instance Fisher's protected least significant difference test (FPLSD) when SNK failed to identify differences due to sub-groups in the means (Thomas, 1973). Where an interaction between time and forage treatment was found comparisons were restricted to between forages within time and between times within forage with comparison-wise type I error rate adjusted using the Bonferroni approximation (Abdi, 2007).

## 3. Results

### 3.1. Earthworm population assessment

After the three years under forage treatment, differences were found in earthworm abundance and biomass across treatments ( $P = 0.002$ ;  $P < 0.001$  respectively; Table 2), with the white clover treatment having greater abundances than the others ( $P < 0.05$ ). Furthermore, the community assemblage of the three earthworm functional groups differed between season ( $P < 0.001$  Epigeics;  $P = 0.008$  Endogeics and  $P < 0.001$  Anecics; Table 2), with higher endogeic and anecic counts and lower epigeic counts in spring. Anecic earthworm abundance was greater ( $P = 0.027$ ) in the white clover treatment, than in the ryegrass 200 N plot ( $P < 0.05$ ) with the other forages intermediate. Neither epigeic, nor endogeic functional groups varied among treatments ( $P = 0.132$ ;  $P = 0.202$  respectively) and MANOVA analysis found that overall for the three groups there was only a tendency for forage treatment to affect community assemblage ( $P < 0.10$ ) (see Table S1 for full community assemblage). There were no significant interactions between treatment and season for total earthworms, biomass or the individual functional groups.

### 3.2. Nematode population assessment

The abundance of nematodes did not differ among forages ( $P = 0.176$ ) or season ( $P = 0.277$ ) (Table 3), ranging between 38 and  $51 \text{ g}^{-1}$  DM soil. Overall, bacterial and fungal feeding nematodes dominated all treatments. The abundance of fungal feeders ( $P = 0.005$ ) and herbivorous nematodes ( $P < 0.001$ ) did differ between treatments with greater numbers of fungal feeders in the clovers and chicory treatments compared to the ryegrass treatments, whilst the herbivores had the greatest abundances in the

**Table 2**  
Earthworm abundance under different forage treatments sampled in autumn and the following spring. Analysis of results using a split plot ANOVA with SNK superscript letters to signify  $P < 0.05$  differences between forages; d.f. for comparisons between F  $\times$  S means excluding those within Forage are shown in parentheses.

	Sample (S)	Forage (F)					Mean	Effect	Prob	s.e.m.	Within forage s.e.m
		Low N	200 N	Chicory	White clover	Red clover					
Total (n m <sup>-2</sup> )	Autumn	402.8	419.4	413.9	819.4	500.0	511.1	F	<b>0.002</b>	81.7	
	Spring	275.0	347.2	425.0	686.1	402.8	427.2	S	0.111	49.6	
	Mean	338.9 <sup>a</sup>	383.3 <sup>a</sup>	419.4 <sup>a</sup>	752.8 <sup>b</sup>	451.4 <sup>a</sup>		F $\times$ S	0.887	113.3 (26.8)	110.9
Epigeic (n m <sup>-2</sup> )	Autumn	101.8	164.3	174.8	298.1	254.0	192.6	F	0.132	0.854	#
	Spring	17.1	18.8	19.7	11.7	15.9	16.6	S	<b>&lt;0.001</b>	0.774	
	Mean	52.4	76.4	80.8	113.8	104.3		F $\times$ S	0.266	1.492 (25.6)	1.730
Endogeic (n m <sup>-2</sup> )	Autumn	236.4	150.7	147.5	270.4	177.4	194.2	F	0.202	1.335	\$
	Spring	187.1	261.2	264.1	451.6	282.9	284.4	S	<b>0.008</b>	0.600	
	Mean	211.2	203.3	202.6	356.3	227.8		F $\times$ S	0.251	1.638 (22.6)	1.341
Anecic (n m <sup>-2</sup> )	Autumn	31.0	9.3	22.6	68.0	24.4	28.9	F	<b>0.027</b>	0.942	#
	Spring	46.5	34.4	107.4	148.0	94.5	81.4	S	<b>&lt;0.001</b>	0.374	
	Mean	38.4 <sup>ab</sup>	20.6 <sup>a</sup>	58.6 <sup>ab</sup>	104.6 <sup>b</sup>	54.8 <sup>ab</sup>		F $\times$ S	0.171	1.112 (20.7)	0.836
Total (g m <sup>-2</sup> )	Autumn	112.4	158.8	187.1	400.4	225.3	206.8	F	<b>&lt;0.001</b>	0.863	¥
	Spring	88.7	96.4	185.2	238.1	162.6	149.1	S	<b>0.072</b>	0.795	
	Mean	100.2 <sup>a</sup>	125.7 <sup>ab</sup>	186.3 <sup>b</sup>	314.0 <sup>c</sup>	192.7 <sup>b</sup>		F $\times$ S	0.766	1.525 (25.4)	1.778

\$. # and ¥; s.e.m.'s apply to means expressed as  $\sqrt{y+66}$ ,  $\sqrt{y+11}$  or  $\sqrt{y}$  respectively.

two ryegrass treatments (Table 3). There was also a trend for bacterial feeders ( $P = 0.074$ ) to be found in greater numbers in the ryegrass treatments; although omnivores and predator abundance was similar across treatments. There were seasonal differences in both fungal and herbivorous nematode functional groups, with both having greater numbers in the spring compared to the autumn ( $P = 0.031$  Fungal;  $P = 0.008$  Herbivores), although there were no forage  $\times$  season interaction effects.

### 3.3. Microarthropod populations

An average of 35,000 ( $\pm 3200$ ) microarthropods per m<sup>2</sup> were extracted across all treatments and both sampling periods; nearly 60% were mites and 35% Collembola, whilst the other 5% were classified as "other" invertebrates. The abundance of both the Collembola and the mite orders did not differ among forage

treatments (Table 4). There were differences however, between the two sampling periods, with greater abundance of both Collembola ( $P = 0.041$ ) and mites ( $P = 0.051$ ) in the spring sampling compared to the autumn. Two collembolan superfamilies did show significant differences between treatments (Poduromorpha and Symphypleona); whilst two did not (Entomobryomorpha and Neelipleona (Table 5a)). The detritivorous Poduromorpha had greater abundances in the alternative forages; whilst the herbivorous Symphypleona had larger abundances in the ryegrass 200 N (Table 5a). The three main mite lineages did not differ in abundance across treatments (Table 5b) however there was a trend towards greater numbers of the predatory Mesostigmata in red clover ( $P = 0.061$ ), and a greater abundance of Prostigmata in the ryegrass low N ( $P = 0.076$ ). Both the Mesostigmata ( $P = 0.027$ ) and the Prostigmata ( $P = 0.015$ ) differed across sampling period (Table 5b), with Mesostigmata being found in greater numbers in the autumn 2012,

**Table 3**  
Nematode abundance (count per g soil DM) under different forage treatments sampled in autumn and the following spring. Analysis of results using a split plot ANOVA with SNK superscript letters to signify  $P < 0.05$  differences between forages; d.f. for comparisons between F  $\times$  S means excluding those within Forage are shown in parentheses.

	Time (S)	Forage (F)					Mean	Effect	Prob	s.e.m. <sup>#</sup>	Within forage s.e.m
		Low N	200 N	Chicory	White clover	Red clover					
Total	Autumn	36.2	46.4	34.8	41.6	37.6	39.2	F	0.176	0.239	
	Spring	44.1	51.1	40.4	45.3	42.3	44.6	S	0.277	0.260	
	Mean	40.1	48.7	37.6	43.4	39.9		F $\times$ S	0.999	0.475 (23.5)	0.581
Bacterial	Autumn	13.2	20.5	14.5	12.5	12.3	14.4	F	<b>0.074</b>	0.220	
	Spring	17.3	15.5	10.8	10.6	10.8	12.8	S	0.450	0.202	
	Mean	15.2	18.0	12.6	11.5	11.5		F $\times$ S	0.748	0.388 (25.4)	0.452
Fungal	Autumn	9.9	12.1	11.5	17.2	15.6	13.1	F	<b>0.005</b>	0.184	
	Spring	12.0	13.8	19.5	23.2	20.4	17.6	S	<b>0.031</b>	0.168	
	Mean	11.0 <sup>a</sup>	13.0 <sup>ab</sup>	15.2 <sup>abc</sup>	20.1 <sup>c</sup>	18.0 <sup>bc</sup>		F $\times$ S	0.835	0.323 (25.5)	0.376
Herbivores	Autumn	7.0	7.0	3.5	4.7	4.1	5.2	F	<b>&lt;0.001</b>	0.092	
	Spring	10.2	15.0	5.8	7.5	3.9	8.0	S	<b>0.008</b>	0.130	
	Mean	8.5 <sup>c</sup>	10.6 <sup>d</sup>	4.5 <sup>a</sup>	6.0 <sup>b</sup>	4.0 <sup>a</sup>		F $\times$ S	0.334	0.225 (20.6)	0.290
Omnivores	Autumn	3.3	3.4	2.8	4.0	3.4	3.4	F	0.156	0.094	
	Spring	2.5	3.9	2.3	2.5	3.7	3.0	S	0.429	0.105	
	Mean	2.8	3.7	2.6	3.2	3.6		F $\times$ S	0.766	0.191 (23.2)	0.235
Predator	Autumn	2.3	2.3	1.9	2.7	1.9	2.2	F	0.580	0.154	
	Spring	1.3	2.5	1.5	1.1	3.1	1.8	S	0.353	0.104	
	Mean	1.7	2.4	1.7	1.9	2.5		F $\times$ S	0.325	0.225 (26.9)	0.232

#; Applies to means expressed as  $\sqrt{y}$ .

**Table 4**

Microarthropods (count m<sup>-2</sup>), analysis of results using a split plot ANOVA with superscript letters to signify *P* < 0.05 differences between forages; d.f. for comparisons between F × S means excluding those within Forage are shown in parentheses. Simpson's 1-D based on counts for all microarthropods extracted (Collembola and mites main groups, Coleoptera larvae, Diptera larvae, Enchytraeidae, earthworms, spiders, Hemiptera, Coleoptera, Chilopoda, Diplopoda, Diptera, Pauropoda, Protura, Symphyla and Thysanoptera).

	Time (S)	Forage (F)					Mean	Effect	Prob	s.e.m.	Within forage s.e.m
		Low N	200 N	Chicory	White clover	Red clover					
Total	Autumn	32,803	30,957	29,022	28,176	26,349	29,377	F	0.215	0.0581	\$
	Spring	48,745	55,884	26,145	33,611	42,184	39,888	S	<b>0.037</b>	0.0410	
	Mean	39,987	41,593	27,546	30,773	33,339		F × S	0.517	0.087 (27.0)	
Collembola	Autumn	7164	9176	11,418	12,724	11,291	10,255	F	0.690	9.20	#
	Spring	13,831	18,726	12,287	13,331	17,074	14,953	S	<b>0.041</b>	6.66	
	Mean	10,226	13,530	11,848	13,026	14,033		F × S	0.625	13.99 (27.0)	
Mites	Autumn	23,060	34,206	11,500	18,768	26,002	17,605	F	0.079	12.66	#
	Spring	34,241	19,463	16,745	16,530	13,016	24,054	S	<b>0.051</b>	7.47	
	Mean	28,375	26,318	13,999	17,631	18,953		F × S	0.241	17.31 (26.1)	
Other	Autumn	2871	2316	1285	1486	1197	1723	F	<b>0.042</b>	0.0768	\$
	Spring	1706	1972	1120	1721	957	1441	S	0.396	0.0629	
	Mean	2213 <sup>b</sup>	2137 <sup>b</sup>	1199 <sup>a</sup>	1599 <sup>ab</sup>	1070 <sup>a</sup>		F × S	0.893	0.1256 (26.5)	
Simpson's 1-D	Autumn	0.634	0.722	0.726	0.759	0.729	0.714	F	<b>0.041</b>	0.0261	
	Spring	0.583	0.672	0.676	0.691	0.728	0.670	S	<b>0.026</b>	0.0125	
	Mean	0.609 <sup>A</sup>	0.697 <sup>B</sup>	0.701 <sup>AB</sup>	0.725 <sup>B</sup>	0.729 <sup>B</sup>		F × S	0.807	0.0327 (23.6)	

\$; s.e.m.'s apply to means expressed as log<sub>10</sub>(y).

#; s.e.m.'s apply to means expressed as √y.

a, b; Means differ at *P* < 0.05 based on FPLSD (SNK test fails to find differences due to sub-groups).

A, B; Means differ at *P* < 0.05 based on SNK test.

**Table 5a**

Collembola superfamilies (count m<sup>-2</sup>), analysis of results using a split plot ANOVA with SNK superscript letters to signify *P* < 0.05 differences between forages; d.f. for comparisons between F × S means excluding those within Forage are shown in parentheses.

	Time (S)	Forage (F)					Mean	Effect	Prob	s.e.m.	Within forage s.e.m
		Low N	200 N	Chicory	White clover	Red clover					
Entomobryomorpha	Autumn	5313	6291	7059	6359	6486	6288	F	0.770	7.61	#
	Spring	11,257	13,399	7686	7991	10,333	10,023	S	<b>0.026</b>	5.94	
	Mean	8009	9513	7369	7152	8298		F × S	0.683	12.09 (26.8)	
Poduromorpha	Autumn	498	569	2384	5298	4472	2060	F	<b>0.008</b>	8.54	#
	Spring	699	585	3515	3201	3498	2178	S	0.821	3.97	
	Mean	594 <sup>ab</sup>	577 <sup>a</sup>	2922 <sup>ac</sup>	4184 <sup>c</sup>	3970 <sup>c</sup>		F × S	0.604	10.60 (23.1)	
Neelipleona	Autumn	95	33	268	925	33	108	F	0.357	4.12	#
	Spring	114	683	389	209	519	482	S	<b>0.014</b>	2.92	
	Mean	104	254	326	504	203		F × S	0.485	6.19 (27.0)	
Symphypleona	Autumn	1070	1908	1134	568	962	1088	F	<b>&lt;0.001</b>	2.97	#
	Spring	1555	2959	294	626	945	1112	S	0.935	3.05	
	Mean	1301 <sup>a</sup>	2405 <sup>b</sup>	646 <sup>a</sup>	597 <sup>a</sup>	953 <sup>a</sup>		F × S	0.364	5.66 (24.2)	

#; s.e.m.'s apply to means expressed as √y.

**Table 5b**

Mite subgroups (count m<sup>-2</sup>), analysis of results using a split plot ANOVA with superscript letters to signify *P* < 0.05 differences between forages or between times within forage (comparisons are only within columns and within rows (not between columns)); Means within columns or within rows with differing superscripts differ with experiment-wise type I error rate of 0.05. d.f. for comparisons between F × S means excluding those within Forage are shown in parentheses.

	Time (S)	Forage (F)					Mean	Effect	Prob	s.e.m.	Within forage s.e.m
		Low N	200 N	Chicory	White clover	Red clover					
Mesostigmata	Autumn	4115 <sup>ab</sup>	5328 <sup>ab</sup>	4418 <sup>ab</sup>	5485 <sup>ab</sup>	5393 <sup>ab</sup>	4931	F	<b>0.061</b>	5.12	#
	Spring	3477 <sup>ab</sup>	5518 <sup>ab</sup>	2121 <sup>a</sup>	2333 <sup>a</sup>	6500 <sup>b</sup>	3800	S	<b>0.027</b>	2.48	
	Mean	3789	5423	3166	3743	5934		F × S	<b>0.043</b>	6.45 (23.7)	
Oribatid	Autumn	252	477	244	33	0	140	F	0.125	4.94	#
	Spring	172	974	296	74	423	329	S	0.196	3.30	
	Mean	210	704	269	51	106		F × S	0.568	7.18 (26.9)	
Prostigmata	Autumn	18,561	13,228	11,821	10,740	7430	12,092	F	0.076	13.34	#
	Spring	30,087	26,797	8755	16,196	17,841	19,130	S	<b>0.015</b>	7.27	
	Mean	23,978	19,420	10,231	13,328	12,074		F × S	0.310	17.60 (25.3)	

#; s.e.m.'s apply to means expressed as √y.

whilst the Prostigmata was found in greater numbers in spring 2013. The interaction between sample time and forage was significant for the Mesostigmata only ( $P = 0.043$ ; Table 5b), this is due to inconsistent changes in population abundance between forages across the two sampling times. The abundances of Mesostigmata in white clover and chicory were numerically lower in the spring than in the autumn ( $P > 0.05$ ), whilst abundances for the other forages were similar.

The abundance of “other” invertebrates did differ among forages ( $P = 0.042$ ; Table 4), with the ryegrass treatments having significantly more “other” invertebrates than either red clover or chicory. This was due to the large numbers of Hemiptera and Thysanoptera in the ryegrass treatments compared to the other forages (Table S1), both of which are herbivorous. There was however no difference between sampling dates for other invertebrates. Simpson's index of diversity found significant differences in community composition across treatments ( $P = 0.041$ ; Table 4). Ryegrass low N had significantly lower levels of species diversity compared to the two clovers and ryegrass 200 N with chicory being intermediate. There was also significantly less diversity in the spring sampling period ( $P = 0.026$ ; Table 4). There were no forage  $\times$  season interaction effects.

#### 4. Discussion

Maintaining a healthy soil food web is known to increase agricultural productivity (DuPont et al., 2009), monitoring how the individual invertebrate orders are affected by the different forage crops, provides an indication of the impact caused by a change in agricultural practice. Scientists are still elucidating the linkages between biodiversity and ecosystem functioning (Lavelle et al., 2006). However, the findings here show the influence forage type has over soil fauna community assemblages, linking the differences in abundance to the characteristics of each agricultural forage crop. Our results agree with the hypothesis that a change in soil habitat (plant type) influences the environment to the extent the faunal biodiversity residing there were affected.

Initial cultivation is likely to have reduced the heterogeneity of the field site (Hendrix et al., 1986), this allowed the impact of the four different forages to take effect. All plots were located next to each other within the same field, indicating that the differences in soil faunal assemblage were due to the different forages. Three years after establishment changes in the soil habitat, differences in root architecture and nutrient availability should become apparent (White et al., 2013). Dispersal rates vary dependent on soil faunal group, e.g. Oribatid mites have been found to migrate 1–8 m annum<sup>-1</sup> (Lehmitz et al., 2012), whilst earthworms can move more than 1 m day<sup>-1</sup> (Caro et al., 2013). Reproductive rates also vary between invertebrate groups, e.g. Oribatid mites can take up to five years to complete a life cycle, whereas Prostigmata mites can take <3 months (Krantz and Walter, 2009), anecic earthworms live up to 10 years, whilst epigeic earthworms live for only 1 year (Sherlock, 2012). These differences in dispersal and reproductive cycles, required the experimental forages to remain for three years before the soil faunal populations were assessed. It is also likely these differences in reproductive rates are why there are differences in abundance of some of the faunal groups across the two seasons measured. The period of growth and harvest cuts within this experiment, also directly relates to the ‘normal’ lifespan of the forages within agriculture, as farmers utilise these forages as short term lays over similar timescales. There were no differences in soil minerals and trace elements between forage treatments (apart from Mn, which was higher in the ryegrass treatments) (as reported in Rhymes et al., 2015).

It has been shown that soil biodiversity loss and simplification of communities impairs multiple ecosystem functions, including

plant diversity, decomposition and nutrient retention and cycling (Wagg et al., 2014). Therefore, there is a real possibility that the change in forage species could lead to changes in ecosystem function – leading to knock-on effects to crop yields. Earthworms are often reported to reflect food availability (Curry et al., 2008), greater abundance increases organic matter consumption, and improves soil structure (Blouin et al., 2013) and leads to greater nutrient flux and an improved soil health. Our results are partly in agreement with Van Eekeren et al. (2009), with greater abundance of earthworms and lower proportion of herbivorous nematodes in white clover only swards compared to ryegrass only swards (Table 2). Earthworm burrows created by anecic species are used by plants as preformed channels for root growth (Ehlers et al., 1983), therefore a forage crop which promotes anecic earthworm abundance is potentially going to increase the productivity within subsequent crops.

Soil animal groups have been found to be negatively affected by the intensity of agriculture (fertiliser inputs/crop rotation) (Ponge et al., 2013); with, intensively managed grassland (high inputs of inorganic fertiliser, increased tillage) promoting bacterial feeding organisms, whilst extensively managed grassland (low input, organic fertiliser, minimum tillage) promoting fungal feeding organisms (De Vries et al., 2012). Our results agree with this to a certain extent, except the ryegrass that received minimal N input over the preceding 3 years did not have significantly different populations from the ryegrass that had received 200 kg N ha<sup>-1</sup> annually, indicating that the forage type maybe more important than the fertiliser regime. Here, the greatest differences were found between the ryegrass treatments and the legumes, confirming other hypotheses regarding the introduction of legumes being a key influence over how soil biota function, promoting soil structure, water retention, biodiversity and C and N storage (Murray et al., 2012).

Herbivorous invertebrates can reduce crop yields, however monitoring the yields of these pure swards prior to this study (Marley et al., 2013a; Rhymes et al., 2015) found the dry matter yields to be representative of other experimental grassland studies although different between treatments. Our results indicate that a higher number of herbivores are found within the ryegrass treatments compared to the clovers and chicory (Tables 3 and 5a), suggesting herbivores are favouring the ryegrass treatments, possibly due to it acting as a refuge or food source. All forages within this experiment have had the same level of disturbance and management, indicating that the herbivores are preferentially increasing in abundance within the ryegrass forages.

There were seasonal effects found for the different organisms between the two sampling periods but this was likely due to the lifecycle of the organisms and/or environmental conditions. For example, earthworms did not show seasonal effects in abundance, but data on the individual functional groups showed seasonal effects, with a higher abundance of epigeics in the autumn of 2012. This finding may have been due to this year having the wettest summer on record in the UK (Met Office, 2012) which favoured the rapid reproduction of epigeics (Sherlock, 2012) in comparison to the slower cycles for endogeics and anecics. In addition, fungal and plant feeding nematodes had higher abundances in spring. It is known that different nematode groups are seasonally variable (Bernard, 1992) so this increase is likely due to the increase in new growth in spring, seasonal patterns having been shown to reflect which organisms are actively feeding at the time of sampling (Elfstrand et al., 2008).

Overall, our results confirm our hypothesis that differences in forage rooting system favour different functional groups of soil fauna. Plants with extensive root systems (ryegrasses) appear to favour herbivorous invertebrates; whilst those forages that are

more mycorrhizal, favour fungivores, however, further work is necessary to confirm this association. Reductions in arbuscular mycorrhizal fungi (AMF) due to fertilisation or seasonality (Gamper et al., 2004) are likely to reduce faunal populations of fungivores within the forage treatment, due to the reduced reliability of food source. Fungal to bacterial (F:B) ratios have been found to decrease with increasing N application, with higher fungal biomass with lower N levels (De Vries et al., 2006). However F:B ratios have many pitfalls if using these as your only indicator (Strickland and Rousk, 2010). Potentially nematode functional group results like these here, could be used as a useful indicator of F:B dominance within microbial communities, as the results are not relying on the growth or biomass of the microbial community (Strickland and Rousk, 2010); focussing on a higher trophic level ameliorates these pitfalls to some extent.

Previously our understanding of the link between different forage crops and soil biota had been difficult to establish because of the complex nature of soil food webs. Here, we have begun to elucidate the different effects plants have on soil biology in the field. Nevertheless, this experiment does not conclusively show whether it is the direct effects of the different forages causing these differences, or whether it is the indirect effects of the different forages changing the soil environment through different rooting systems, or exudates that have led to these differences. Further work can build on this study, investigating how the overall quality and productivity of the forage crop is affected by these differences in soil food web assemblage. The findings of this paper highlight the linkages between some forage crops and specific organism groups and showed that overall different forages will change the soil food web composition even if management techniques and environmental variables remain the same.

## 5. Conclusion

Our study compared belowground soil food webs in order to assess the effect of growing different forage crops. The findings of this study showed that soil faunal diversity and abundance were different under the different forage crop types. Our findings suggest the clovers and chicory provide greater ecosystem services due to the increased abundance of some faunal groups (earthworms, Poduromorpha Collembola etc) which increase decomposition, redistribution of nutrients and promote the N and C cycle. Further work now needs to determine whether it is the direct effects of the different forages on soil fauna or whether it is the indirect effects of the forages changing the soil environment differently. Overall, our findings show that linking soil fauna with plant type should be a consideration when implementing sustainable farming methods.

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## Appendix A. Supplementary material

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.soilbio.2015.08.036>.

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