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Long-Term Fertiliser Regimes have both Direct and Indirect Effects on Arthropod Community Composition and Feeding Guilds

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ABSTRACT

Vegetation species composition and structure are known to affect taxonomic composition and life history characteristics of arthropod communities. Soil conditions alter vegetation composition and structure, and thus soils have indirect effects on arthropods. Whilst grassland management affects soil properties, and hence vegetation, the direct effects of soil on arthropod communities within the sward is less clear. We used a long-term hay meadow experiment to assess both direct and indirect effects of various fertiliser regimes on arthropod community composition and feeding guilds. Arthropods were sampled via pitfall traps and sweep nets, then analysed using principal components and redundancy analyses (RDA) to determine relationships between soil properties, vegetation community, forage quality and arthropod community. Vegetation community composition, measured by the first vegetation principal component, was used as a constraining variable in partial RDA, to estimate direct effects of soil on the arthropods. Variance partitioning quantified the relative roles of vegetation and soil on the arthropod community. Our results indicate that available soil nitrogen and carbon-nitrogen ratios are important determinants of arthropod community composition. Once the effects of the vegetation were removed it was found the soil acidity and the available potassium altered arthropod community composition. Further research is required to determine the mechanisms by which these soil properties affect arthropod communities.

Keywords: Soil properties, feeding guilds, vegetation community, grasslands, nutrient additions, diversity, long-term experiments.

INTRODUCTION

Arthropods are affected by vegetative species composition, vegetation structure and grassland management practices. The plant species composition has been shown to affect the species richness and abundance of arthropods (cf. Altieri 1999; Woodcock and Pywell, 2010; Ebeling *et al*. 2012; Badenhausser *et al*. 2015; Dassou *et al*. 2016; Huelber *et al*. 2015). Schaffers *et al*. (2008) reported that plant species composition was the best predictor for an assemblage of different arthropod taxonomic groups and families. Abiotic factors affect the species composition of the vegetation, and therefore the vegetation structure, which in turn affects arthropod assemblages. For example spiders and ground beetles have been shown to be affected by ground cover (Rushton *et al*. 1989; Eyre *et al*. 1990) and butterflies prefer a sward with a non-uniform height (Jerrentrup *et al*. 2014). A heterogeneous sward will contribute generally to arthropod species richness and abundance (WallisDeVries *et al*. 2002). Another aspect of the vegetative structure is the microclimate, which has also been determined as being important in relation to arthropods. Rae *et al*. (2006) investigated its relationship with epigeal herbivores and predators and found that shorter swards generally had higher temperatures than tall swards, with a direct effect of temperature on the herbivorous community. These are all direct effects of the vegetation on arthropods.

The management regime on a grassland governs the vegetation composition and structure (Socher *et al*. 2012). A frequent hay cut, intensive grazing and high levels of fertiliser application (particularly nitrogen and phosphorus) can all result in reduced species richness (Manning *et al*. 2015), whereas a hay cut once or twice a year, light grazing and fertiliser use can result in increased species richness of vegetation and hence arthropods (Birkhofer *et al*. 2008; Mazalova *et al*. 2015). Fertiliser use alters soil nutrient status and acidity, which in turn will have a direct effect on the vegetation species composition, forage quality and structure (Crawley *et al*, 2005), and therefore an indirect effect on arthropods. However, soil may also have direct effects on arthropods once the effects of vegetation species composition have been removed. These direct effects may include soil chemical properties, such as acidity, available nitrogen and soil moisture which have been shown to have a direct effect on Collembola (Salmon *et al.* 2002; Silvertown *et al*. 2006) and soil microbial communities (Sun *et al*. 2015), but little is known concerning any direct effects of the soil on the community composition of larger arthropods commonly used in ecological studies (e.g. Coleoptera, Diptera, Hymenoptera and Hemiptera).

The aims of this paper are to: 1. Determine the effects of fertiliser regimes on the arthropod and vegetation community composition in a long-term hay meadow experiment. Specifically, to assess the differences in the community composition of the arthropods and vegetation as a result of the long-term management regimes, and to determine whether there are any differences in the main feeding guilds. 2. Assess the indirect effects of the soil characteristics on the arthropod community composition, as measured through soil properties and resultant changes in forage chemistry. 3. Determine if there are any direct effects of the fertiliser regimes and soil properties on arthropod community composition, after accounting for differences in the vegetation species composition. 4. Quantify any direct effects of the vegetation species composition and soil properties on the arthropod community.

METHODS

Site description

The site of Palace Leas is part of the 220 hectare mixed agriculture Cockle Park farm in Northumberland, United Kingdom, grid ref NZ202912 (55^o 13’ N, 1^o 41’ W). Palace Leas itself is a set of 14 unreplicated plots which are directly adjacent to each other, each plot is approximately 0.18ha in area (120m x 15m); see Hopkins *et al.* (2009) and Kidd *et al.* (2017) for diagrams of the experimental setup, and detailed history of the site. The experimental plots are bounded by a narrow buffer strip, hedgerow and road at the southern end, the eastern edge has a strip that contains mainly grass and thistle before a crop of barley. To the north there is another field of barley directly adjacent to plot 14 and to the west a grass track then a ditch. The field adjacent to the ditch is used for light sheep grazing. The plots have had the same treatments applied every year since 1896, with the exception of plot 14 which was introduced in 1976 to reflect the amount of nutrients used at that time. The plots are cut once a year in late June/early July and the yield measured. An annual vegetation survey is also conducted in late May/early June each year. Light sheep grazing occurs in September and October each year. There is a weather station on site.

Eight plots were used (Appendix A, Table 1) that all had the same rotation system with annual organic (FYM) and/or mineral (NPK in different combinations) fertiliser application (excluded plots had 2 to 4 yearly rotations). The farmyard manure (FYM) is applied in February/early March (complete by 5th March in 2015) and is supplied by the farm for plot 1 (FYM & NPK) and plot 2 (FYM). Nitrogen is in the form of sulphate of ammonia for plot 7 (N only) and plot 13 (NPK low), whilst plot 14 (NPK high) receives ammonium nitrate and plot 1 (FYM & NPK) receives sodium nitrate. Phosphorus is in the form of triple super-phosphate, and applied to plot 1 (FYM & NPK), plot 8 (P only), plot 13 (NPK low) and plot 14 (NPK high). Potassium is applied as muriate of potash to plot 1 (FYM & NPK), plot 9 (K only), plot 13 (NPK low) and plot 14 (NPK high). The mineral fertilisers are applied late March/April; in the survey reported here it was 8th April 2015.

Field sampling

Arthropods were collected by both sweep nets and pitfall traps, and for the purposes of sampling, each plot was sub-divided into four quarter plots (30m x 15m). Sweep net samples were collected between the 8th and 23rd June 2015, before the hay was cut in the first week of July. During this period the mean daytime temperature was 12.98°C, mean overnight temperature 10.36°C, mean daily precipitation 0.9mm and mean sunshine 6.53 hours per day. The sweep nets used were triangular head, with an arm length of 30cm. A rectangular pattern (approximately 2m x 12m) was swept in each quarter plot, to produce a single sample in each quarter plot, providing four samples per plot. The pitfall samples were collected between the 17th and 27th August 2015, seven weeks after the hay cut. Pitfall traps are most effective in sampling epigeal arthropods, whilst sweep nets would be less useful to survey the post-hay cut fauna, therefore different methods were used for the two time periods. This sampling period had a mean daytime temperature of 16.5°C, mean overnight temperature 14.3°C, mean daily precipitation 3.6mm and mean sunshine 6.13 hours per day. The traps were polypropylene cups with an opening diameter of 7.50cm and a depth of 13cm, they were filled with a saturated salt (NaCl) solution plus a small amount of detergent to break the surface tension. The pitfall traps were placed in the centre of each quarter plot, with their tops flush to the surface and left for seven days before collection, again resulting in four samples per plot.

The sward height (SH) for each plot was measured, during the sweep net sampling period, using a sward stick (Stewart *et al*. 2001); the maximum height of the sward was measured at 6 random locations within and around area sampled with the sweep nets to give a mean maximum height for each quarter plot. Vegetation species composition was sampled by percentage cover, with four 1m quadrats equally spaced, along a central transect in each plot. This data was collected two weeks prior to the arthropod sweep net sampling period. The hay is cut annually, with each quarter plot cut separately. A subsample was taken from each of the quarter plots to determine the different aspects that relate to forage quality: moisture content (MC), dry weight (DW), percentage nitrogen (N%), percentage carbon (C%) and the carbon- nitrogen ratio (CN.1) of the hay (Appendix A – Table 2). The cut was done the week following the arthropod sample collection. Soil samples were collected in September 2014 to determine, soil pH (pH), soil organic carbon content (SOC), soil carbon-nitrogen ratio (CN), soil nitrogen (SoilN), phosphorus (SoilP) and potassium (SoilK) (Appendix A – Table 3).

Identification and data analysis

All Insecta were identified to taxonomic family and allocated to a feeding guild, using Insects of Britain and Western Europe (Chinery, 2012). Where families contained more than one feeding guild they were allocated to the predominant (by numbers of individuals) feeding guild. The main taxon falling into this category was the Hemiptera-Miridae which contains both herbivorous and predatory species, but most Miridae collected in this study belonged to herbivorous species (only a small number of specimens were found, and the second author identified all these to species). Some taxa belong to multiple guilds during their life-history and in these cases taxa were allocated to the main life-history stage collected in the study; as a result of the sampling methods used and timing of sampling, these were primarily adults. Unlike the Insecta, all Araneae belong to the same feeding guild (predators), and therefore were counted but not identified to family. Note that this decision has minor implications for the interpretation of the analyses (see Discussion). Only arthropods of approximately >2mm length were identified.A full breakdown of the arthropod taxa and allocated feeding guilds is presented in Appendix B Table 1.

Differences in the numbers of arthropod individuals as a result management were determined through linear models (one-way ANOVA) with plot treatment as the explanatory variable. Arthropod species community composition and vegetation composition (percentage cover) data were analysed using ordination techniques via principal components analysis (PCA) and redundancy analysis (RDA) and partial redundancy analysis (pRDA) to address the four main aims of the paper, using the R vegan package (Oksanen, 2015):

1. PCA is an unconstrained linear ordination technique, and was used to address the first aim of the study, i.e. to understand the effects of the long-term management regimes on the arthropod and vegetation community composition, and provide a convenient summary of the relative similarity in species composition. Vegetation PC1 was used as a summary variable in subsequent analyses (3 and 4 below) to partial-out the effects of vegetation species composition.
2. RDA is a constrained ordination methods, such that the positions of the response variable (the samples by taxa matrix) is constrained by environmental factors, so that the sample scores are linear combinations of the environmental variables. RDA was used to address the second study aim, to measure the effects of soil chemistry, and its knock-on impacts on forage properties, on the arthropods. The arthropods by samples matrix was used as the response

variable in the RDA, with a matrix of soil chemistry and forage quality by samples providing explanatory (constraining) variables.

3. pRDA differs from RDA in that partial (conditioning) explanatory variables can be incorporated into the analysis, so that the effects of other explanatory variables are easier to understand. This is particularly useful where it is suspected that some explanatory variables might have a major effect on the response, and would therefore obscure the effects of the other explanatories. It is well-established that vegetation species composition has a major effect on grassland arthropod communities, but a key aim of this study was to measure the effects of the fertiliser regimes after accounting for their effects on vegetation species composition. We therefore used pRDA with soil properties as standard explanatory variables, but vegetation species composition (as measured by vegetation PC1 sample scores) as a partial explanatory variable.
4. Variance partitioning (Borcard *et al*, 1992) uses a series of linked pRDA analyses to quantify the individual and joint effects of several explanatory variables; in this study we used it to determine the relative importance of the vegetation species composition and soil properties to quantify their relative effects on the arthropod communities.

As the raw data for the sweep net, pitfall trap and vegetation communities were dominated by a small number of families the data was standardised using the Hellinger transformation (Legendre and Gallagher, 2001) prior to principal components analysis and constrained analysis being performed. Log and square-root transformations are common alternatives transformations, particularly for percentage data; however both our arthropod and vegetation community datasets were skewed by a small number of very abundant taxa, and the Hellinger transformation proved more robust in avoiding undistorted ordination diagrams. Statistical significance of explanatory variables in RDA and pRDA was tested through Monte Carlo permutation tests, using 999 permutations (Oksanen, 2015).

RESULTS

Overall summary of arthropod and vegetation samples

A total of 3542 arthropod specimens were captured across 69 different families, with Diptera having the greatest number of individuals at 2436 (68.8%), and the largest number of families at 42. The most abundant were Scathophagidae with 1550 individuals found mainly in the sweep net samples. The majority of these were the yellow dung fly, *Scathophaga stercoraria* (L. 1758). The next most numerous were the Aranae with 408 specimens and the Carabidae with 181, though both these were found mainly in the pitfall samples. A breakdown of the samples by taxonomic group and fertiliser treatment is provided in Appendix A with a complete list of all families and their functional traits in Appendix B.

The most species-rich vegetation community was in the P-only treatment (25 species) whilst the lowest was the N-only treatment (6 species). The plot with nitrogen-only additions was dominated by grass species (5) with only one forb, whereas, in the other plots the number of grass species and number of forb species were similar. The British National Vegetation Classification (NVC: Rodwell, 1991) shows both plots with FYM additions being classified as *Lolium perenne* leys and related grasslands, *Lolium perenne-Alopecurus pratensis* sub-community, the control and phosphorus only additions were *Cynosurus cristatus-Centaurea nigra* grassland*, Lathyrus pratensis* sub-community, both these classes are mesotrophic grasslands. The remaining plots were classified as *Festuca ovina-Agrostis capillaris-Galium saxatile* grassland, *Holcus lanatus-Trifolium repens* sub-community, which are calcifugous grasslands and montane communities.

Overall effects of fertiliser treatments on arthropod feeding guilds

Sweep net samples were dominated by decomposers, with 74% of 2565 individual arthropods across all plots. Of the 1906 decomposer individuals, 1070 (56%) were recorded from the two plots that received farmyard manure (FYM & NPK; FYM plots). Herbivores predominated in those plots that only received NPK (NPK low and NPK high): 185 (51%) of the total of 363 herbivores individuals. Pollinators were most abundant on the two plots that received FYM (48% of all pollinators), with the remainder fairly evenly spread between the other plots. Only 36 saprophytic (fungus-feeder) individuals were collected, two thirds of these were in the two plots treated with the FYM, and none were found in those with the low NPK and high NPK plots. Linear models indicated no significant difference between the total number of individual arthropods caught by sweep netting between the plots, based on the 4 samples per plot (F = 0.532, p = 0.805, df = 7).

Pitfall samples were all dominated by predators, with 73% of 977 individual arthropods across all plots, but there was relatively little difference in the numbers of predators in each plot. Most herbivores were found on plots receiving only NPK treatment (low NPK and high NPK plots): 29% of all herbivore individuals. Decomposers were mainly associated with the two treatments receiving farmyard manure (FYM & NPK; FYM plots): 47% of decomposer individuals. Most parasites were found on the plot that only received phosphorus (P only): 42% of parasite individuals. Linear models indicated no significant difference between the total number of individuals caught by pitfall traps between the plots, based on the 4 samples per plot (F = 0.119, p = 0.996 df = 7).

1. Principal components analysis to determine treatment effects on arthropod and vegetation communities

Arthropods - Sweep samples PCA results

The first two axes of the principal components analysis (PCA) of the standardised sweep-net data explained 52% of the variance, with axis 1 at 34%. There is a gradient from decomposers to herbivores along the PC1 axis (Fig. 1), with the plots receiving the FYM treatments, the saprophytes and the decomposers being associated with each other. To a lesser extent, the plot receiving nitrogen-only was also associated with the saprophytes and the decomposers. The plots receiving the NPK treatments showed an association with some herbivores, especially Chrysomelidae (leaf beetles) and Delphacidae (leaf bugs). The first principal component was mainly associated with soil C:N ratio ($R^2 = 0.497$) and soil N ($R^2 = 0.496$), whilst the second principal component was weakly associated with soil P ($R^2 = 0.311$).

FIGURE 1

Arthropods - Pitfall samples PCA results

The first two PCA axes for the standardised pitfall trap samples explain 57% of the variance, with 38% on axis 1 (Fig. 2). Decomposers were associated primarily with low PC1 scores, and herbivores and saphrophytes had higher scores, but trends along PC1 were less obvious than for the sweep samples. The arthropod composition in the plots with nitrogen-only and phosphorous-only are similar, indicated by their proximity on the PCA plot, as are those in the potassium-only and NPK low. Whilst there is an association between the FYM & NPK plot and the phosphorous-only plot along the first principal component, on the second principal component the FYM & NPK plot is very different from all the other plots. The first principal component was strongly associated with soil N ($R^2 = 0.576$) whilst there was a weak association between PC2 and sward height ($R^2 = 0.257$).

FIGURE 2

Vegetation – PCA results

Fig. 3 summarises the vegetation species and experimental plots from the PCA (PC1 65%, PC2 18%). Grass species were distributed across the PCA graph, but the forbs are concentrated around the centre of the graph, suggesting a greater degree of variation in the species composition of the grasses between the different experimental plots than for the forbs. The first principal component was strongly correlated with the available soil phosphorus ($R^2 = 0.942$) and to a lesser extent with the C:N ratio ($R^2 = 0.796$) and the soil nitrogen ($R^2 = 0.514$), showing a gradient from high soil phosphorus to high C:N ratio. This coincides with a gradient from those plots with FYM additions to the plots with K-only, N-only plots and the control. The second principal component had a correlation with soil pH ($R^2 = 0.543$) which coincides with the phosphorus additions and the control, at high pH and the NPK high and N only additions at low pH.

FIGURE 3

2. Constrained ordination via RDA to determine effects of environmental variables on the arthropod community

RDA of sweep net samples

When the sweep net samples were constrained by the soil data using RDA (fig.4a), the factors which most strongly affect the arthropod community are the C:N ratio and available soil nitrogen, though permutation tests showed no significance (F=1.41, p=0.181, df=7). The soil nitrogen, soil organic carbon content and the available phosphorus have similar effects, and are most closely associated with the FYM only additions and the Sepsidae family of Diptera (see Fig. 1). The C:N ratio is negatively associated to these three soil properties.

Constrained RDA with the vegetation structure and vegetation chemistry (fig.4b) shows a gradient from height to C:N ratio for the first principal component which also coincides with a FYM to NPK variance between the plots (F=1.174, p=0.391, df=7). The second principal component is related to the moisture content of the vegetation with the arthropod community in the plots with the potassium and phosphorus additions preferring the drier vegetation. The factors showing the strongest effect on the arthropods are the height of the vegetation and its moisture content.

Constraining by soil properties and the PC1 scores from the plant community PCA (fig. 4c), the soil C:N ratio and the vegetation PC1 scores had similar effects on the arthropod community, though the available soil nitrogen, which was negatively associated with the vegetation had an equally strong effect. The overall RDA permutation test was non-significant (F=1.0365, p=0.518 df = 7). The arthropod community in the plots with K-only and P-only treatments were associated with high vegetation PC1 scores, which itself was associated with larger amounts of vegetation species such as *Festuca rubra*, *Anthoxanthum odoraturm* and *Agrostis capillaris* (refer back to Fig. 3a). Conversely, Fig 4c indicates that FYM-treated plots were associated with low vegetation PC1 scores (and higher soil N, SOC and soil P), and from Fig 3a this would suggest plants such as *Poa trivialis*, *Agrostis pratensis* and *Bromus hordeaceus* were characteristic of the vegetation that was generating the largest effects on the arthropods.

RDA of pitfall samples

Constrained RDA with soil data (fig. 5a) indicated the strongest soil variables for the pitfall trap samples were available soil nitrogen and the C:N ratio, i.e. the same as for sweep net samples, and also non-significant (F=1.162, p=0.428, df=7). A high C:N ratio is closely associated with the arthropod community from the plot with NPK low additions, and to a slightly lesser extent the plot with the K only additions.

When constrained by the vegetation structure and vegetation chemistry, the C:N ratio and dry weight of the vegetation are the strongest factors (fig. 5b). The community in the NPK low and K only plots are aligned with the higher C:N ratio and prefer the lower nitrogen content (F=0.823, p=0.760, df=7). The dry weight of the vegetation is the strongest explanatory variable, with a gradient from the control plot to FYM & NPK.

Constraining by the vegetation species composition (vegetation PC1 score) and soil properties on the epigeal community (fig. 5c) showed similar patterns to that from the sweep samples, with both vegetation PC1 and soil C:N ratio being highly correlated and important predictors (F=1.9422, p=0.077, df = 7). High C:N ratio and vegetation PC1 scores were associated with sites receiving NPK (high or low) or K-only; again, cross-reference back to Fig. 3a suggests the epigael arthropods in these samples are from vegetation dominated by *F. rubra, A.odoratum* and *A. capillaris*.

3. Partial constrained ordination via pRDA to determine effects of environmental variables on the arthropod community after accounting for vegetation composition

pRDA of sweep net samples

The effects of the soil properties on the sweep net arthropods, once the effects of the vegetation community have been removed by using vegetation PC1 as a partial variable (Fig. 4d) indicated that the soil K and soil pH had the largest effects on the arthropod community composition. The effects of soil N and C:N ratio on the arthropods was reduced once the vegetation community composition had been partialled-out (compare Figs. 4a and 4d).

The constraining variable soil K was, as might be expected, associated with the experimental plot receiving the K-only treatment (Fig. 4d). The arthropods in this treatment were predominantly characterised by an abundance of Curculionidae (see Fig. 1). High soil pH was related particularly to the plots receiving FYM & NPK, and P-only, and conversely low pH with the NPK-low plot (by extending the pH arrow in Fig 4d backwards). Cross-reference with Fig. 1 suggests that arthropods characteristic of alkaline conditions include Chloropidae, Scathophagidae and Sciaridae. High soil N and SOC were characteristic of the N-only treatment; this is not characterised by any particular group of arthropods.

FIGURE 4

pRDA of pitfall net samples

Community composition of arthropods collected by pitfall trap is most strongly affected by the soil K, N and pH (fig. 5d) once the effects of the vegetation had been removed via partial RDA (vegetation PC1 scores). The control plot was associated with high soil pH and N, which from Fig. 2 suggests Lonchopteridae, and Cynipidae are affected by available nitrogen. As might be expected, soil K was strongly positively associated with the K-only treatment. The FYM & NPK treatment point is approximately equidistant from potassium and soil pH arrows in Fig 5d, suggesting that both constraining variables are important. Cross-reference with Fig. 2 suggests Chrysomelidae were particularly associated with this treatment plot.

FIGURE 5

4. Variance partitioning via pRDA to quantify direct and indirect effects

The main direct effect identified by variance partitioning on the species composition of the sweep-net arthropods was the vegetation species composition, at 27.1%. In contrast, the vegetation had a smaller effect on pitfall (epigeal) arthropods caught via pitfall traps (13.8%), whilst the soil chemistry had a larger effect on epigeal arthropods in comparison to the sweep net arthropods (11.6% vs 8.2%, Table 1). The joint amount of variation explained by both the vegetation and the soil was relatively low at approximately 4% in both cases. However, irrespective of sampling method, over 50% of the variation in the arthropod community was unexplained by either soil chemistry or vegetation species composition.

TABLE 1

DISCUSSION

This study has investigated the relationships between the vegetation species composition, structure, forage quality, soil properties, and the arthropod community, in a long-term hay meadow experiment. Our results demonstrate that the management has both direct (changes in soil properties) and indirect effects (mediated through the vegetation) on the arthropod community composition.

The vegetation species community had a strong relationship with the soil in terms of available phosphorus, soil acidity and the carbon-nitrogen ratio, and the arthropod community had a strong relationship with nitrogen, both in terms of the available nitrogen in the soil and the nitrogen content of the vegetation. This agrees with previous studies by La Pierre and Smith (2016) who suggested that nutrient additions, especially nitrogen, increased the abundance of herbivorous insects in the mesic prairies of North America. The arthropod herbivores, especially Chrysomelidae and Delphacidae, are more abundant where the C:N ratios are higher and therefore the relative amount of nitrogen is lower. Whilst this result does not agree with the results of many previous studies (cf. Wang *et al*. 2006,) there have been some instances where nitrogen additions and therefore a lower C:N ratio have been shown to either reduce or make no change to herbivore abundance (Bethke *et al*. 1998, Casey and Raupp, 1999). Rae *et al.* (2006) found that the microclimate in the sward, especially soil and air temperature, can have a direct effect on the herbivore community; in our study treatments with high N resulted in greater vegetation growth, which may have affected the microclimate. The relationship between nitrogen and the arthropod community is due to the effects that the nitrogen availability and C:N ratios have on the vegetation and is therefore an indirect relationship.

The long term addition of nitrogen-only fertilisers produced a similar arthropod community structure to that of the plots which had farmyard manure additions, even though the vegetation showed more similarity with the plots that had the NPK additions. This may be because a layer of organic matter, 6cm thick, had developed over the top of the soil at Palace Leas as a result of the low acidity caused by the nitrogen additions (Shiel and Rimmer, 1984). So the results for the nitrogen-only plot show that it is the resultant soil properties that have influenced this arthropod community more than the vegetation. Short term nitrogen only fertiliser use may not have the same results.

The biggest differences in the arthropod communities were between the plots with FYM treatment and those with NPK treatments. Plots receiving FYM & NPK nutrient additions showed many similarities with those that only received FYM and was very different to the mineral fertiliser (NPK) plots, suggesting that farmyard manure may be a dominant factor determining the species composition of arthropod communities, especially decomposers and saprophytes. Whilst the vegetation species composition was also affected by FYM application, the differences between the plots were not as obvious as for the arthropod communities.

Most previous studies have investigated the joint effects of vegetation and environmental factors on arthropod communities (Birkhofer *et al*. 2008; Crist and Peters, 2014; Manning *et al*. 2015). In contrast, we have attempted to identify the environmental factors that have both direct and indirect effects on the arthropods, depending on how they are mediated by the vegetation. Removal of the vegetation community composition, via partial RDA, indicated that arthropod community composition, collected by sweep net, was directly affected by soil pH and the available potassium in the soil. The epigael community was also affected by the available nitrogen in the soil. These environmental factors had not shown any importance until the vegetation species community data had been removed, suggesting that the effects of the carbon-nitrogen ratio, and soil organic carbon on the arthropod community were indirect and due to the effects that these factors had on the vegetation, whereas soil pH, available potassium and for the epigael community, available nitrogen, do have direct effects. This may be in relation to the fact that they somehow affect the microclimate within the sward, especially with the volatilisation of nitrogen (Brown *et al*. 2010). The main direct and indirect factors are summarised in Fig. 6.

FIGURE 6

When variance partitioning was applied the vegetation species composition had a larger influence on the arthropod community than the soil properties, and the soil properties have more of an effect on the epigael community than the sweep net community. These results are in agreement with many other studies (Curry 1994; Harte *et al.* 1996; Schaffers *et al*. 2008). However, there is a high proportion which remains unexplained. This may be due to the structure of the vegetation and the microclimate created by it. These results do however, show a direct effect from the resultant soil properties due to the different fertiliser regimes, with soil nitrogen being the most influential factor.

A novel aspect of this research is that the direct effects of the soil conditions on arthropod community composition have been identified, as well as the readily measured vegetation effects. Whilst the latter has been reported widely in the scientific literature (*cf*. Schaffers *et al*. 2008; Woodcock and Pywell, 2010), there are relatively few studies of the former. The exact causal mechanisms for the soil directly affecting the arthropod community composition cannot be ascertained directly in the current study, but they might be associated with factors such as microclimate (e.g. differences soil moisture) and nutritional value of plants (e.g. available nitrogen to arthropod herbivores).

One weakness of this study relates to the fundamental design of the experiment when it was originally create in the late 19th Century. None of the treatments are replicated in separate plots, and so technically the four sets of samples we have collected within each plot could be viewed as ‘pseudo-replicates’. The design of the experiment would have been better if there had been replicate plots and a block design, but such concepts in statistical theory and experimental design were not fully understood at the time. Nevertheless, an advantage of utilising an experiment such as this is that it provides a unique opportunity to gain insights into the effects of treatments when applied continuously over long time-periods. Vegetation changes that arise from short-term experiments of only a few years duration differ significantly from long-term studies. For example, Kidd *et al.* (2017) in a parallel study of the soil and vegetation at Palace Leas demonstrated that plots receiving only artificial fertilisers, with the exception of the high-NPK treatment, became dominated by lower yielding, acid-tolerant species. Our results indicate that such long-term changes in the soil and vegetation will inevitably have knock-on effects on the arthropods.

A second limitation is that there is no single sampling method that is suitable for foliar and epigeal arthropods, and both techniques have biases, are affected by meteorological conditions, vegetation density etc. It must be recognised that arthropods sampled from within one plot may have originated from neighbouring plots, and this will make it more difficult to detect true treatment effects on the taxonomic composition of the arthropods. Finally, the interaction between vegetation biochemistry, structure and arthropods is complex, and affected by feeding type. For example, it has long been established that higher plant nitrogen generally increases vulnerability to arthropod herbivores (McNeil and Southwood, 1978), but the role of predatory/parasitic species is more complex, as they may detect the arthropod prey directly, or volatiles (including induced-defence chemicals) released by the vegetation when under attack by herbivores (Poelman and Dicke, 2014). Many arthropod guilds are affected by changes in vegetation structure (Woodcock and Pywell, 2010) The different management regimes affected vegetation structure, which has major impacts on some groups of arthropods, especially for some of the larger species within the Araneae (Mcnett and Rypstra, 2000) .

In summary, the soil properties and the vegetation have indirect effects, arising from the different fertiliser regimes, on the species composition of the arthropod community, whilst the addition of farmyard manure provides direct effects on the decomposers and saprophytes. This study has been done in a single year, on a long-term experiment, and it should be emphasised that it may take many years before these separate mechanisms can be identified.

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