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LONG-TERM CHANGE IN VEGETATION AND SOIL MICROBIAL COMMUNITIES DURING THE PHASED RESTORATION OF TRADITIONAL MEADOW GRASSLAND

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Running title: meadow plant diversity and belowground properties

Summary

1. Restoration of high plant species diversity to sites where it has been reduced by intensive grassland management requires identification of appropriate management regimes. Understanding the combinatorial effects of management on above-ground vegetation and below-ground microbial communities will inform management prescriptions on how best to increase plant diversity, restore rare vegetation types and achieve agri-environmental objectives.

2. Changes in vegetation and soil microbial community structure are described from the second phase of a 1990-2004 field trial that investigates the interacting effects of fertiliser and farmyard manure (FYM) treatments imposed after 1998; in the context of previous hay cut date and seed addition treatments.

3. Hay cut date was the main factor influencing plant species composition in phase 1, whereas FYM was the dominant factor in phase 2.

4. *Poa trivialis* and *Lolium perenne* increased in abundance with FYM application, particularly in combination with mineral fertiliser, and particularly in 2002 after the 2001 Foot and Mouth epidemic. The lowest Ellenberg fertility scores were associated with absence of FYM and mineral fertiliser but with addition of seed.

5. Highest plant species diversity in phase 2 was associated with seed addition and the absence of mineral fertiliser, an effect that had probably persisted from phase 1. Progressive development of the target traditional meadow vegetation occurred through phase 2, particularly when FYM was applied in the absence of mineral fertiliser.

6. Fungal:bacterial (F:B) ratios, a measure of changes in the relative abundance of fungi and bacteria in the microbial community, generally increased from 1996-2004 and were particularly high in the seed addition treatments, and in the absence of fertiliser. Here the high F:B ratios were associated with species (including legumes) typical of traditionally managed mesotrophic grassland in northern England.

7. *Synthesis and applications.* These results demonstrate that biodiversity goals for upland meadows need to plan beyond the typical 5-10 year management agreement period of agri-environment schemes. Combination treatments, in which seed addition is vital, alongside appropriate fertiliser, farmyard manure, hay cut date and grazing regimes, are needed for grassland restoration. However, even after 14 years the most effective treatment combinations had still not restored the target species composition and diversity. The demonstrated change in soil microbial communities, linked to the growth of legumes, might be important to facilitate future increases in plant diversity.

Key-words: farmyard manure, fertiliser, long-term trial, belowground properties, secondary succession.

Introduction

Restoration schemes for enhancing plant species diversity in agricultural grassland have featured in UK and European agri-environment schemes since 1985. In the UK this has been through Countryside Stewardship and Environmentally Sensitive Area (ESA) schemes; replaced on 3 March 2005 by Environmental Stewardship. On 1 September 2006 this latest scheme covered 3.1M ha, with 23000 live agreements in place representing first year payments of more than £105M. This is about 30% of UK agricultural land eligible for such payments. However, despite the periodic revision of management prescriptions their effectiveness has recently been questioned (Kleijn & Sutherland 2003, Whitfield 2006). Knop *et al.* (2006) have demonstrated significantly higher plant species diversity in Swiss meadows under agri-environment schemes although they acknowledged that the difference may have been present prior to the implementation of the scheme, confirming that rigorous evaluation needs to be incorporated into agri-environment schemes (Kleijn & Sutherland 2003). In the meantime, data on the long term consequences of different management treatments for mesotrophic grasslands, such as hay cut dates, fertiliser addition and grazing regimes, plus an understanding of some of the underlying ecological processes, should be used to identify appropriate management treatments for enhancing diversity and to inform policy on the likely timescales. Experiments across a range of grassland types have included tests of single treatments such as grazing regimes (Smith & Rushton 1994). Tests of biological tools have included the use of the hemi-parasite *Rhinanthus minor* to debilitate competitive grasses (Pywell *et al.* 2004) and the sowing of seed when converting arable land to grassland (Pywell *et al.* 2002) and when enhancing diversity in improved ryegrass swards (Smith *et al.* 2000).

Such experiments have identified some of the key management features and ecological processes that control plant species diversity in mesotrophic grassland. They have also shown that it can take many years to successfully increase plant diversity and return grass swards to some semblance of their traditional character. Five-year experiments begun in the 1990s, and subsequently extended, will not fully influence management prescriptions for many years; with yet further delays when putting experimental results into farming practice. Therefore, concern about the effectiveness of grassland agri-environment schemes can be expected, especially when experiments indicate that the ecological problem is more intractable than first thought and requires investigation of ecological mechanisms through field trial and mesocosm experiments (Bardgett *et al.* 2006).

Here we present the phase 2 results from a long-term meadow grassland experiment begun in 1990 at Colt Park, in North Yorkshire, England, UK. It was devised to determine the best combination of management practices for the restoration of plant species diversity to agriculturally improved grassland. Specifically, the experiment tested the effects of three grazing, three hay cut date, two fertiliser and two seed addition treatments on the plant species composition of a *Lolium perenne-Cynosurus cristatus* grassland (MG6) (Rodwell 1992; *Lolio-Cynosuretum cristati* grasslands in European terminology). The first phase (1990-98) demonstrated that all the main treatments produced small but significant changes to plant species diversity (Smith *et al.* 2000), with a particularly large increase occurring with a combination of autumn cattle and spring sheep grazing, a 21 July hay cut date and addition of seed of many species. A large decrease in diversity occurred when, in the absence of autumn cattle grazing, hay was cut on 14 June and fertiliser was also applied. *Rhinanthus minor* spread to most plots after its introduction in the seed treatment, being particularly abundant in

1996 in all treatment combinations that included autumn grazing, no mineral fertiliser and a 21 July hay cut. Populations of >40 plants m^{-2} were associated with low hay yields. This first phase of the trial thus demonstrated that the species composition of the meadow was particularly affected by combinations of management treatments.

Addition of seed was important for the restoration of species diversity as the soil seed bank was very similar in species composition to the above-ground vegetation of agriculturally improved meadows and contained few extra species that could contribute to an increase in sward diversity (Smith *et al.* 2002). Also there were many fewer species than in the soil seed bank of a traditionally managed meadow. The hemiparasite *Rhinanthus minor* (hay rattle) was thought to have been an important driver of the observed vegetation change as it is known to reduce the dominance of competitive grasses (Joshi *et al.* 2000, Pywell *et al.* 2004) across a range of residual soil fertility and farmyard manure applications (Bardgett *et al.* 2006). This suggests great potential for its use in the restoration of species-rich grassland (Davies *et al.* 1997, Pywell *et al.* 2004). In addition, hay rattle has been shown to increase rates of soil nitrogen mineralization (Bardgett *et al*. 2006) and change other soil microbial properties. When seed was added to unfertilised plots cut on 21 July, increased species richness had been observed at Colt Park by 2000, primarily through an increase in legumes, stress-tolerant and stress-tolerant-ruderal plant strategists, and associated with increase in fungal abundance in soil (Smith *et al.* 2003). Our interest in the soil microbial community was based upon the possible significance of changes in the abundance of soil fungi and soil fungal:bacterial (F:B) ratios for the restoration of plant species diversity. Reductions in soil F:B ratios have been linked to intensive management and especially the use of mineral fertiliser, whereas it has been suggested that increases in this ratio are associated with more efficient nutrient cycling, increased abundance of mycorrhizal fungi, and enhancement of plant species diversity (Donnison *et al*. 2000; Bardgett *et al.* 2001; Bardgett & McAlister 1999; Grayston *et al.* 2004). These shifts in microbial community structure resulting from intensive management may be reversible, albeit over long time-scales, and the introduction of particular plant species into the sward might accelerate this reversal, acting to promote fungal growth in soil (Smith *et al.* 2003; Bardgett *et al.* 1996).

By 1998 it was evident that differences in grazing regime and hay cut date were major drivers of vegetation change in the Colt Park experiment. Consequently, advice for agri-environment and other conservation schemes for the diversification of grassland, was that hay should be cut in mid-July and the regrowth (aftermath) should be grazed with cattle in the autumn. It was also evident that the second phase (1998-2004) needed to include an additional farmyard manure (FYM) treatment to address a developing management and research issue. FYM is an inevitable waste product of livestock rearing under cover in barns but it has some value as a fertiliser. It is spread on meadows in winter and early spring at various rates. This waste disposal supplies a variable amount of nutrients for plant growth, with an average nutrient composition of 6 kg nitrogen, 3.5 kg phosphate and 8 kg potash per tonne FYM (Simpson & Jefferson 1996; Ministry of Agriculture, Fisheries and Food 1994). The maximum rate of FYM application prescribed for meadows in the Pennine Dales ESA was 12 t ha^{-1} (Ministry of Agriculture, Fisheries and Food 1992) i.e. a possible 72 kg ha^{-1} N, 42 kg ha^{-1} P and 96 kg ha^{-1} K to meadows. However, only 20% of these nutrients are thought to be available to the crop following spring FYM application – or 0.5, 3.4 and 7.7 times the amounts of N, P and K supplied by the standard ESA maximum acceptable rate of mineral fertiliser, and used here at Colt Park.

After the 1998 hay harvest the trial was continued on the autumn and spring grazing treatment plots and the other grazing treatments (two thirds of the experiment) were abandoned. The 21 July cut date treatment was then applied to all remaining plots to investigate the effects of restoring traditional hay cut dates where early- and late-haycuts had been applied for 8 years.

A new FYM treatment was applied from 1999 by subdivision of the original plots. These smaller plots were more than twice the size of the $4m^2$ quadrats used for sampling, with past treatments producing distinctive changes at this sampling scale. Whilst the wind blown seed of *Rhinanthus minor* was expected to disperse between plots (Coulson *et al*. 2001) other species had demonstrated considerable fidelity to the sown treatment in phase 1. Elsewhere, sharp boundaries between experimental plots under different treatment combinations had demonstrated the dominant effect of treatments as environmental sieves for plant colonisation (Shiel & Hopkins 1991). The success of seed sowing in the first phase indicated that more seed of missing species could be sown in the original seeded plots at the start of the second phase.

The split-plot design in phase 2 (Table 1) allowed past 1990-98 treatments to be continued though phase 2, as well as providing for tests of interactions between the various treatment types and allowing an interpretation of the FYM results against a combination of pre-treatments. The following predictions were tested.

1. The magnitude of the effects of past management treatments on plant species composition will change over time, with the additional nutrients in FYM increasing the abundance of competitive species.
2. Any post-1998 changes in vegetation and soil microbial community would be dependent on the sward composition in 1998.

3. Returning to a 21 July hay cut date after 8-years of 14 June or 1 September cuts would, within 6 years, produce a grassland similar to that cut on 21 July throughout the experiment.
4. The already demonstrated effects of the management treatments on the soil microbial community (as expressed as the relative abundance of fungi and bacteria), would have increased by 2004.
5. Correlation between plant species and soil microbial community properties would continue to develop.
6. The sowing of additional species typical of traditional meadows would successfully reintroduce them into the sward in phase 2 even if inclusion in the original 1990-92 seed addition had failed to do so.

Methods

The field trial at 300m altitude at Colt Park meadows in the Ingleborough National Nature Reserve (National Grid reference SD775782, latitude 54^{o}12'N, longitude 2^{o}21'W) was on *Lolium perenne-Cynosurus cristatus* grassland (MG6) (Rodwell 1992), on a shallow brown-earth soil (pH 5.1) over limestone of moderate-high residual fertility (15 mg P_2O_5 l^{-1}). Management prior to 1990 consisted of autumn and spring grazing, mineral fertilizer application and a 21 July cutting date for hay.

From 1998 autumn and spring grazing was continued where it had been applied to three 12 x 36-m paddocks (0.043ha), with livestock having free access to the plots. Grazing regimes were (1) 30 beef cattle intensively grazed (10.9 cattle ha^{-1}) during four 3-day to 5-day periods from mid-July to late-August; (2) 150 cross-bred lambs throughout August (7.5 lambs ha^{-1}); (3) ten beef cattle throughout October (0.9 cattle ha^{-1}); (4) 40 young sheep (less than one-year-old) (2 sheep ha^{-1}) from October to mid-

March and (5) 200 ewes (6.9 sheep ha^{-1}) during lambing from mid-April to mid-May. This grazing regime was generally followed each year but differed in 2001 when grazing with cattle was not possible because of an epidemic of Foot and Mouth disease in England.

In phase two, a 21 July hay cut was applied across the trial (Table 1) with a BCS Commander 630ws mower (supplied by Tracmaster Ltd, Burgess Hill, UK), with a 1.3-m wide reciprocating cutter bar. The cut hay from a plot was dried on that plot, turning it once to assist drying and seed dispersal, then removing it from the trial. This mid-May to mid-July period for the growth of the hay crop was typical of pre-1970 agriculture in the N. England uplands (Smith & Jones 1991; Smith 1997). The two levels of the fertiliser treatment were continued with each cut-date plot divided into two 6 x 12-m sub-plots randomly allocated to no fertiliser or 25 kg ha^{-1} nitrogen plus 12.5 kg ha^{-1} phosphorus (P_2O_5) and potassium (K_2O). The fertiliser was a proprietary 20:10:10 NPK brand spread by hand in early May each year. The original fertiliser sub-plots had been further divided into two sub-sub-plots for the phase 1 seed addition treatment. This was continued post-1998 with 15.4 kg ha^{-1} of commercial seed (Emorsgate Seeds) of each of *Lotus corniculatus*, *Briza media* and *Ranunculus bulbosus* sown in August 1998; with 0.5 kg ha^{-1} of *Geranium sylvaticum* seed sown in September 1999. In 1999 each seed treatment was further subdivided into two 6 x 3-m sub-sub-sub-plots and a farmyard manure (FYM) treatment allocated at random to one of each pair (i.e two FYM treatments per sub plot). Treatments of no-FYM and 12 t ha^{-1} FYM were applied in April 1999, then again in November-December from 1999 onwards. The FYM was obtained in March-April each year from a local farm, stored in a covered midden until required the following winter. Three replicates of each treatment combination were split between three blocks.

Vegetation sampling was based on a subdivision of each 18-m^2 plot into a central grid of two 4 m^2 quadrats, with a surrounding 1-0.5m wide boundary. All vascular plant species were identified in these quadrats using nomenclature according to Stace (1991), and their percent contribution to the total vegetation cover was estimated by eye during June or early-July every two years from 1994-2004, before the plot was cut for hay. The soil microbial community was analysed using the phospholipid fatty acid analysis (PLFA) technique (Bardgett, Hobbs & Frostegård 1996), with analyses undertaken in 1996 and 2000 being repeated in July 2004; see Smith *et al.* (2003) for detailed methods.

Redundancy analysis (RDA), available within CANOCO (version 4.5) (Ter Braak & Smilauer 2002), was used to assess the proportion of the variability in plant species composition attributable to each treatment at each 2-year sample period from 1994-2004 (Ter Braak & Smilauer 2002). This tests prediction 1, which was also tested by analysis of the Principal Response Curves (Ter Braak & Smilauer 2002) through phases 1 and 2 within plots that had consistently been grazed in the autumn and spring and cut for hay on 21 July. Here treatments were compared over time against the species composition of the pre-1990 management regime, i.e. plots that had just received mineral fertiliser, were cut for hay on 21 July but had not been treated with FYM or had seed added.

A repeated measures analysis of Variance (SAS statistical software) was used to investigate changes over time (1996-2004) and the effects of the interaction of time with the applied treatments on the species richness, Ellenberg fertility and the similarity of the vegetation to a target plant community (prediction 3). Epsilon values were calculated according to the method of Greenhouse & Geisser (1959) to adjust the degrees of freedom based on the divergence of the covariance matrix from homogeneity.

The target plant community was *Anthoxanthum odoratum – Geranium sylvaticum* grassland (MG3b) (Rodwell 1992) and the similarity was assessed with the TABLEFIT coefficient (Hill 1996). Upper and lower target values were defined from a random set of pseudoquadrats for subcommunities of MG3, MG4, MG5, MG6 and MG7 vegetation using methods described by Smith *et al.* (2003). These lower and upper limits were respectively one standard deviation below and above the mean value for a plant community in this data set. Ellenberg fertility was assessed for each quadrat from the sum of the cover values for each species, weighted by Ellenberg's fertility index, recalculated for British conditions by Hill *et al.* (2000).

ANOVA was also used to assess treatment differences in 2004 for the PLFA variables, including derived variables such as the ratio of fungal-to-bacterial fatty acids (F:B). The F:B ratio was used as an indicator of changes in the relative abundance of fungi relative to bacteria (Bardgett, Hobbs & Frostegård 1996). Fungal PLFA was estimated as the abundance of the fatty acid 18:2ω6 and bacterial PLFA as the sum of the abundance of the fatty acids i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, 17:0cyclo, 18:1ω7 and 19:0cyclo (Frostegård, Bååth & Tunlid 1993). This ANOVA tests prediction 4. As described earlier a repeated measures analysis (using SAS) was used to investigate changes over time (1996-2004) and the interaction of time with the applied treatments on F:B ratios (prediction 4) on plots that had been autumn and spring grazed, cut on 21 July without the addition of FYM throughout the 1996-2004 period. This tests the effects of fertiliser and seed addition on F:B ratios in 1996, 2000 and 2004. Significant interactions between treatments and time would indicate that the response of the vegetation to post-1998 treatments was dependent upon the composition of the vegetation in 1998 (prediction 2). As with the other variables, a test of normality (the Anderson-Darling test) was applied to the residuals of each ANOVA. If this deviated significantly from normality then a suitable transformation was applied to the original data. Interactions between treatments were tested as part of the ANOVA. Prediction 2 was also tested by RDA of the 1998-2004 vegetation, with the characteristics of the 1998 vegetation, defined as the sample quadrat scores on the first four axes of a Principal Components Analysis of the 1998 vegetation data, used as covariables to remove the effects of the phase 1 management treatments. Here the blocks were covariables within which the random permutations were made for the Monte Carlo tests in CANOCO (Ter Braak & Smilauer 2002).

Prediction 5 was tested by RDA of the 2004 plant species cover and soil PLFA data, the latter being used as 'environmental' data with management treatments used as supplementary nominal variables, positioned within the ordination after construction. Presentation of the resultant species-PLFA-treatment triplots was simplified by removing the PLFA variables that made little contribution to the variation in plant species. Prediction 6 was tested by inspection of the biplots to see if the traditional meadow species sown in 1998-99 had appeared in the sward by 2004.

Results

In phase 1, differences in hay cut dates dominated the plant species composition of the vegetation of the autumn and spring grazed plots (Fig.1), but this effect was greatly reduced within two years of imposing a common 21 July hay cut date. The FYM treatment accounted for most of the variability in species composition in phase 2, particularly in 2002, when all applied management treatments together accounted for about 35% of the variation. The impact of FYM was also seen in the Principal Response Curves (Fig. 2) for plots cut on 21 July from 1990-2004. These demonstrate that in the absence of mineral fertiliser the vegetation through phases 1 and 2 included *Ranunculus* *acris, Rhinanthus minor* and *Anthoxanthum odoratum*, plus others that had been sown into the sward with the seed addition treatment. FYM application in phase 2, particularly in combination with mineral fertiliser, was associated with increases in *Poa trivialis* and *Lolium perenne.* Here the species composition was very different from that of the baseline treatment, particularly in 2002.

The repeated measures analysis of all cut date, fertiliser, seed and FYM treatment combinations in phase 2 (2000-2004) demonstrated that greater species richness was separately associated with the addition of seed and the absence of mineral fertiliser (Table 2), and probably persisted from phase 1 (Fig. 3). The similarity of vegetation to the target MG3b plant community increased throughout phase 2 and was greatest where plots had been cut on 14 June in phase 1, when fertiliser was not applied and when seed had been added (Table 2, Fig. 4). Interactions between treatments were demonstrated over time (Table 3), with the sward consistently increasing its similarity to the target vegetation when FYM was applied in the absence of mineral fertiliser and vice versa. These increases stopped in 2002 when both FYM and fertiliser were absent or applied together.

These changes were similar, although in the opposite direction, to those of the Ellenberg fertility scores. Ellenberg fertility was lowest when fertiliser was not applied, and in the later years of phase 2 (Table 2, Fig 5). There were a number of interactions such that Ellenberg fertility scores were high when plots had been cut on 14 June 1990-97 and were low where hay had been cut on 21 July from 1990, in the absence of fertiliser (Table 4). FYM application increased Ellenberg fertility scores where fertiliser had been added in the absence of seed (Table 5). This joint effect was particularly high in 2002 (Table 6).

Fungal:bacterial (F:B) ratios and the amount of fungal PLFA in 2004 were greater in the absence of FYM and mineral fertiliser (Table 7), although there were no significant interactions between these treatments in that year. However, F:B ratios generally increased from 1996 in plots consistently grazed in the autumn and spring, and cut 21 July without FYM addition (Table 8) and there was an interaction between treatments such that F:B ratios were particularly high when seed had been added in the absence of fertiliser and low in the presence of fertiliser (Table 8). There was a significant association (F=32.67, P=0.002) between vegetation and PLFA measures along the first canonical axis of the RDA species-PLFA-treatment triplot (Fig. 6). This axis linked the absence of FYM with an increase in fungal PLFA and higher F:B ratios, and with plant species typical of traditional MG3 grassland, e.g. *Rhinanthus minor*, *Anthoxanthum odoratum* and *Ranunculus acris*. High F:B ratios were also associated with the presence of five species sown into the sward as part of the seed addition treatment. Lower F:B ratios were associated with species typical of improved fertilised grasslands, e.g. *Poa trivialis*, *Alopecurus pratensis* and *Dactylis glomerata*. The seed addition treatment in 1990-92 had been supplemented in 1998 with four additional species, two of which, *Briza media* and *Geranium sylvaticum*, occurred in only one and two sample quadrats in 2004. However, *Ranunculus bulbosus* and *Lotus corniculatus* were more frequent in 2004, with frequencies of 6.9% and 14.6% respectively. *L. corniculatus* was found where FYM had not been used and with other species typical of traditional grassland management (Fig. 6).

Discussion

These results show that the effects of phase 1 management treatments on plant species composition did not continue into phase 2, but changed over time and were predominantly a consequence of FYM application, particularly in combination with mineral fertiliser (Prediction 1). The particularly large increases in *P. trivialis* and *L. perenne* in this combination of treatments in 2002 may have been a response to the Foot and Mouth disease outbreak in England. The field trial site was within the area where livestock movements were restricted, so that cattle were not available for autumn grazing in 2001. Interactions between treatments had a major influence on vegetation in phase 2, just as in phase 1 (Smith *et al.* 2000), despite accounting for a relatively small proportion of the variation in species composition.

There was the possibility that any post-1998 vegetation change related to FYM application might be dependent upon the species composition of the sward at the end of phase 1 (prediction 2). The ANOVA demonstrated that FYM did not have a significant effect on the vegetation as a phase 2 main treatment, but interacted with other treatments to change species composition. The inclusion of time in the fertiliser x FYM treatment interaction might support prediction 2. However, the demonstrated effect of time was not a consequence of linear change through phase 2 but a peak in 2002. This suggests an alternative explanation and we favour the idea that the 2002 change in species composition of the sward was a temporary consequence of the lack of cattle grazing in 2001 due to the Foot and Mouth epidemic. If this was the case then phase 2 vegetation change linked to FYM applications was independent of the sward composition in 1998. This may be due to the relatively small, albeit significant, differences in the vegetation in 1998.

The major effects of hay cut date on vegetation in phase 1 were replaced by a major FYM effect, the effect of cut date on plant species composition progressively declining in phase 2 (Figure 1). However, there were still some significant but small past cut date effects on the similarity of the sward to the target MG3b vegetation (Table 2), and a phase 2 cut date x fertiliser interaction for Ellenberg fertility scores (Table 4). Therefore, whilst the imposition of a common hay cut date across the trial in phase 2 greatly reduced the vegetation differences created in phase 1, it did not completely remove them after 6 years (prediction 3). The 21 July cut date applied to all plots in phase 2 will have facilitated the spread of *Rhinanthus minor* to those plots previously cut on 14 June. This will probably be a major factor in vegetation development and facilitate the post-1998 convergence in species composition through the known hemiparasitic effects of *R. minor* on other species (Bardgett *et al.* 2006) and the possible facilitation of colonisation by other species as demonstrated elsewhere by Pywell *et al.* (2004).

We detected significant differences in the composition of soil microbial communities between management treatments in 2004. In particular, the ratio of fungal-bacterial fatty acids was greatest in the absence of FYM and mineral fertiliser, demonstrating a continued development of the soil microbial community towards fungal dominance under these treatments. This finding is consistent with comparative studies of grassland types, which show that traditional management is associated with fungal-dominated microbial communities with high fungal:bacterial PLFA ratios, whereas intensive management is associated with bacterial dominance of the microbial community and low fungal:bacterial ratios (Donisson *et al*. 2000; Bardgett *et al.* 2001, Bardgett & McAlister 1999; Grayston *et al.* 2004). Furthermore, the values of fungal-to-bacterial ratios detected in these treatments in 2004 were of similar magnitude to those of traditional meadows (Bardgett & McAlister 1999); this indicates that it is possible to convert bacterial dominated soil microbial communities of intensively managed grassland systems to fungal dominated communities, more typical of traditional systems, over 14 years. The increased F:B ratios associated with seed addition in the absence of fertiliser (Table 8) suggests that these below-ground changes might be driven by the above-ground changes in the plant species composition of the sward (prediction 5) (Wardle *et al.* 2004). While this suggestion of a cause-effect link between vegetation and soil microbial community change is not proved by the data presented here, it remains a possible explanation for the demonstrated associations between plant species and soil fatty acids (Figure 6) and the F:B ratios found under different treatment combinations (Tables 7 & 8). Also, previous studies have shown strong associations between different plant species of grassland and their microbial communities (Bardgett *et al.* 1999; Innes, Hobbs & Bardgett 2005), suggesting that changes in the dominance of plant species in the community have the potential to alter microbial community structure of soil. The establishment of *R. bulbosus* and *L. corniculatus* in phase 2, after failure to establish during phase 1 (prediction 6), might be a precursor to further species colonisation and increase in sward diversity, after sowing of additional species in 2006 during phase 3 of this meadow trial. However, other factors may have been influential here, such as non-viable seed in phase 1 and the spread of *R. minor* in phase 2.

These results have management implications in terms of (1) the expected timescale for vegetation change, (2) appropriate treatment combinations, (3) the use of FYM, (4) vegetation response to change of hay cut date, (5) the role of seed addition and (6) the link with soil microbial communities.

(1) Overall, our findings indicate that biodiversity goals for upland meadows need to plan beyond the typical 5-10 year management agreement period of agri-environment schemes. There appears to be a limit to what is rapidly achievable. It has taken 14 years for the apparent fertility (from Ellenberg scores) to decline and the vegetation to become similar to that of the target MG3b community in the most effective treatment combinations, but the species diversity was still well below target. 20-year

management agreements might need to be the minimum expectation for policy planning.

(2) Various types of management are applied to meadow grasslands and it is important to apply a combination of a mid-July hay cut date, autumn grazing with cattle, spring grazing with sheep and no mineral fertiliser. This combination provides a niche for the hemiparasite *R. minor*, with major consequences for the relative abundance of other members of the plant community.

(3) FYM at the rate applied here (12 t ha^{-1} every year in winter or early-spring) is not appropriate. It is particularly deleterious with mineral fertiliser, even when this is at ESA application rates, when it increases the abundance of species associated with high soil fertility.

(4) A return to a mid-July cut date after 8 years of early (mid-June) and late (early-September) hay cuts can rapidly, within 6 years, change the vegetation to a more traditional composition, particularly when there is a clear and close seed supply of *Rhinanthus minor* and maybe other species to occupy new niches.

(5) The addition of seed of missing species is essential for increase in sward diversity. Niches created by appropriate sward management will remain unoccupied by additional species unless they can disperse into the sward naturally or artificially from sown seed or strewn hay. The continued phased sowing of seed is probably a sound long term strategy although the Colt Park experiment does not yet provide direct evidence for this.

(6) We have demonstrated here that soil fungal:bacterial ratios increase over time in the absence of fertiliser and in appropriately grazed and cut plots without FYM. Future increase in species diversity might require further change in soil microbial communities, currently linked to the growth of *L. corniculatus, T. pratense, T. repens*,

A. odoratum, R. acris and *R. minor* at Colt Park. We have no data on the mechanisms involved. We have yet to prove it but grassland restoration might need to be viewed as a long-term succession from species-poor to species-rich grassland that requires facilitation of the fungal component in soil microbial communities. Early-successional facilitator plant species may then provide niches for successful colonisation by mid- and late-successional species to recreate traditional species-rich swards in the long term (>20 years).

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Table 1 Experimental design of the autumn and spring grazed plots 1990-2004.

Phase 1 ANOVA structure (degrees of freedom): cut date (C), 2; mineral fertiliser (F), 1; seed addition (S), 1; CxF, 2; CxS, 2; FxS, 1; CxFxS, 2; residual, 24.

Phase 2 ANOVA structure (degrees of freedom): C, 2; F, 1; S, 1; farmyard manure (M), 1; CxF, 2; CxS, 2; FxS, 1; CxM, 2; FxM,1; SxM, 1; CxFxS, 2; CxFxM, 2; CxSxM, 2; FxSxM, 1; CxFxSxM, 2; residual, 48.

Phase 1: August 1990-1998			Phase 2: August 1998-2004			
Cut date	Fertiliser	Seed	Cut date	Fertiliser	Seed	FYM
C1	F0	S0	C2	F0	S0	M0
						M1
		S1			S1	M0
						M1
	F1	S0		F1	S0	M0
						M1
		S1			S1	M0
						M1
C2	F0	S0	C2	F0	S0	M0
						M1
		S1			S1	M0
						M1
	F1	S0		F1	S0	M0
						M1
		S1			S1	M0
						M1
C3	F0	S0	C2	F0	S0	M0
						M1
		S1			S1	M0
						M1
	F1	S0		F1	S0	M0
						M1
		S1			S1	M0
						M1

Table 2. Phase 2 mean vegetation characteristics associated with main treatments 2000-04, significant differences assessed with repeated measures ANOVA.

(a) Past cut date	14 June	21 July	1 September	F	Df	*P*
MG3b similarity (%)	59.06	57.81	57.00	9.51	2, 4	<0.05
(b) Fertiliser addition	No fertiliser	Fertiliser	F	Df	*P*	
Species richness (spp $4m^{-2}$)	21.4	19.3	12.97	1, 6	<0.05	
Ellenberg fertility	4.57	4.67	84.65	1, 6	<0.001	
MG3b similarity (%)	60.19	55.73	15.42	1, 6	<0.01	
(c) Seed addition	No seed	Seed	F	Df	*P*	
Species richness (spp $4m^{-2}$)	18.7	22.0	147.72	1, 12	<0.001	
MG3b similarity (%)	54.76	61.17	51.10	1, 12	<0.001	
(d) Year	2000	2002	2004	F	Df	*P*
Ellenberg fertility	4.74	4.65	4.48	50.58	2, 86.5	<0.001
MG3b similarity (%)	53.56	59.16	61.16	63.24	2, 93.7	<0.001

Table 3. Phase 2 interaction effects of fertiliser treatments, with FYM over time on MG3b similarity scores; $F_{2, 93.7}=4.13$, $P=0.0198$

	No fertiliser		Fertiliser added	
Year	No FYM	FYM added	No FYM	FYM added
2000	55.7	54.9	50.3	53.3
2002	62.9	60.2	54.9	58.6
2004	62.9	64.4	58.5	58.8

Table 4. Phase 2 interaction effects of fertiliser and past cut date treatments on weighted Ellenberg fertility scores; $F_{2, 6}$=12.41, *P*<0.01

	No fertiliser	Fertiliser added
Cut 14 June 1990-97, 21 July 1998-2004	4.687	4.714
Cut 21 July 1990-2004	4.502	4.662
Cut 1 September 1990-97, 21 July 1998-2004	4.525	4.645

Table 5. Phase 2 interaction effects of fertiliser, seed addition and FYM treatments on weighted Ellenberg fertility scores; $F_{1, 24}$=8.04, *P*<0.01.

	No fertiliser		Fertiliser added	
	No seed	Seed added	No seed	Seed added
No FYM	4.431	4.349	4.441	4.391
FYM added	4.765	4.741	4.992	4.871

Table 6. Phase 2 interaction effects of fertiliser, seed addition and FYM treatments over time on weighted Ellenberg fertility scores; $F_{4, 86.5}$=6.95, *P*=0.0023.

	No fertiliser				Fertiliser applied			
	No seed		Seed applied		No seed		Seed applied	
Year	No FYM	FYM	No FYM	FYM	No FYM	FYM	No FYM	FYM
2000	4.633	4.862	4.562	4.708	4.649	5.017	4.617	4.858
2002	4.349	4.816	4.281	4.864	4.417	5.164	4.296	5.032
2004	4.310	4.618	4.205	4.649	4.258	4.794	4.261	4.723

Table 7 Treatment effects on soil microbial characteristics 2004.

	No FYM	FYM	$F_{1, 48}$	*P*
Fungal PLFA	48.28	39.97	5.23	<0.05
Fungal:bacterial ratio	0.06978	0.05907	4.94	<0.05
	No fertiliser	Fertiliser	$F_{1, 48}$	*P*
Fungal PLFA	48.22	39.98	11.01	<0.05
Fungal:bacterial ratio	0.07163	0.05721	7.18	<0.05

Table 8 Mean fungal:bacterial ratios at 4-year intervals (1996-2004) in plots consistently grazed in autumn and spring, and cut 21 July without FYM addition.

Time effect ($F_{2, 19.9}$=61.418, P<0.001)		
1996	2000	2004
0.0240	0.0381	0.0742

Fertiliser x seed interaction ($F_{1,24}$=11.108, P<0.05)		
	No seed	Seed added
No fertiliser	0.0474	0.0539
Fertiliser added	0.0435	0.0369

Figure 1 Variance in plant species composition attributable to management treatments within Autumn and spring grazed plots 1994-2004. Open diamonds, hay cut date; infilled diamonds, FYM; open squares, fertiliser; open triangles, seed addition; infilled circles, treatment interactions

Figure 2 Principle response curves for the 1994-2004 change in species composition, relative to that in the control treatment (mineral fertiliser addition), within autumn and spring grazed treatments with a 21 July (1990-2004) hay cut date treatment. F, fertiliser applied; S, seed added; M, FYM applied; O, no treatments. Species codes (emboldened codes indicate those species sown into the sward as part of the seed addition treatment): Am, *Achillea* millefolium; Ap, *Alopecurus pratensis*; Ao, *Anthoxanthum odoratum*; As, *Anthriscus sylvestris*; Bh, *Bromus hordeaceus*; **Cm**, *Conopodium majus*; Cc, *Cynosurus cristatus*; Dg, *Dactylis glomerata*; Fr, *Festuca rubra*; Hs, *Heracleum sphondyllium*; Hl, *Holcus lanatus*; **Lh**, *Leontodon hispidus*; **Lc**, *Lotus corniculatus*; Lp, *Lolium perenne*; **Pl**, *Plantago lanceolata*; Pt, *Poa trivialis*; **Pv**, *Prunella vulgaris*; **Ra**, *Ranunculus acris*; Rr, *R. repens*; **Rm**, *Rhinanthus minor*; **Tp**, *Trifolium pratense*; Tr, *Trifolium repens*; Vc, *Veronica chamaedrys*; Vs, *Veronica serpyllifolia*.

Figure 3 1994-2004 change in species richness where 2000-04 treatment differences were significant (Table 2): vertical bars are standard errors; horizontal line at 26.2 represents the target species richness for restoration management; open circles, no fertiliser applied; infilled circles, fertiliser applied; open triangles, no seed added; infilled triangles, seed added.

Figure 4 1994-2004 change in similarity to target MG3b vegetation where 2000-04 treatment differences were significant (Table 2): vertical bars are standard errors; horizontal line at 65 represents the target TABLEFIT score for restoration management; open circles, no fertiliser applied; infilled circles, fertiliser applied; open triangles, no seed added; infilled triangles, seed added; squares, year.

Figure 5 1994-2004 change in Ellenberg fertility score where 2000-04 treatment differences were significant (Table 2): vertical bars are standard errors; horizontal line at 4.79 is the lower threshold for MG6a grassland, the line at 4.67 is the upper threshold for MG3b grassland as targets for restoration management; open circles, no fertiliser applied; infilled circles, fertiliser applied; squares, year.

Figure 6 Redundancy analysis of 2004 plant species composition (solid arrows with small heads), with PLFAs (environmental variables represented by dotted arrows with large heads) and management treatments. The latter supplementary variables are represented by symbols: diamonds, FYM; triangles, past hay cut dates; squares, fertiliser; circles, seed. Only the most important species and PLFA variables are included and PLFA names follow Frostegård *et.al.* (1993). Species codes (emboldened codes here indicate those species sown into the sward as part of the seed addition treatment): Alopprat, *Alopecurus pratensis*; Anthodor, *Anthoxanthum odoratum*; Bromhord, *Bromus hordeaceus*; Cardprat, *Cardamine pratensis*; Dactglom, *Dactylis glomerata*; Festrubr, *Festuca rubra*; Holclana, *Holcus lanatus*; **Lotucorn**, *Lotus corniculatus*; Lolipere, *Lolium perenne*; **Planlanc**, *Plantago lanceolata*; Poa triv, *Poa* *trivialis*; **Ranuacri**, *Ranunculus acris*; Ranurepe, *R. repens*; **Rhinmino**, *Rhinanthus minor*; **Trifprat**, *Trifolium pratense*; Trifrepe, *Trifolium repens*.

Figure 1

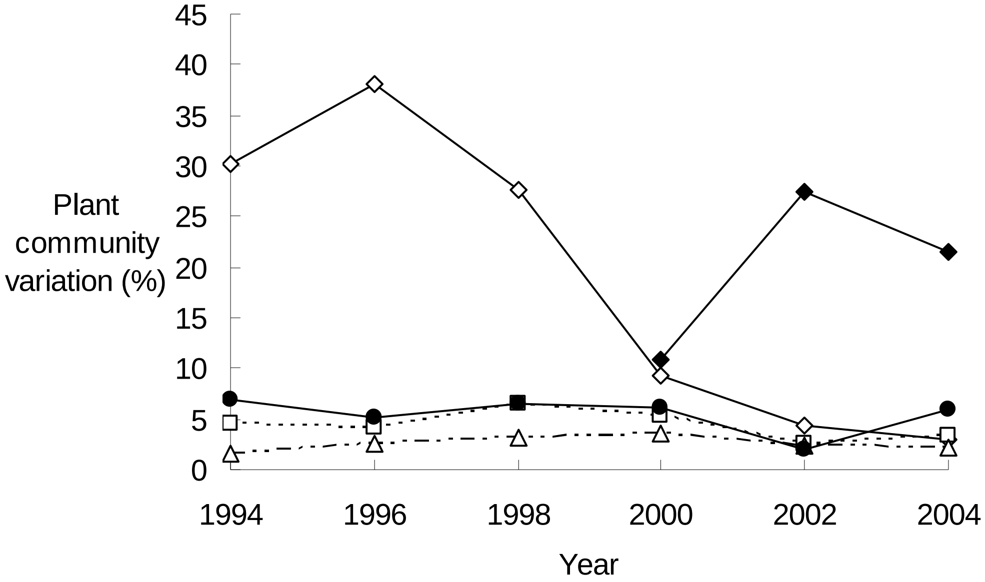
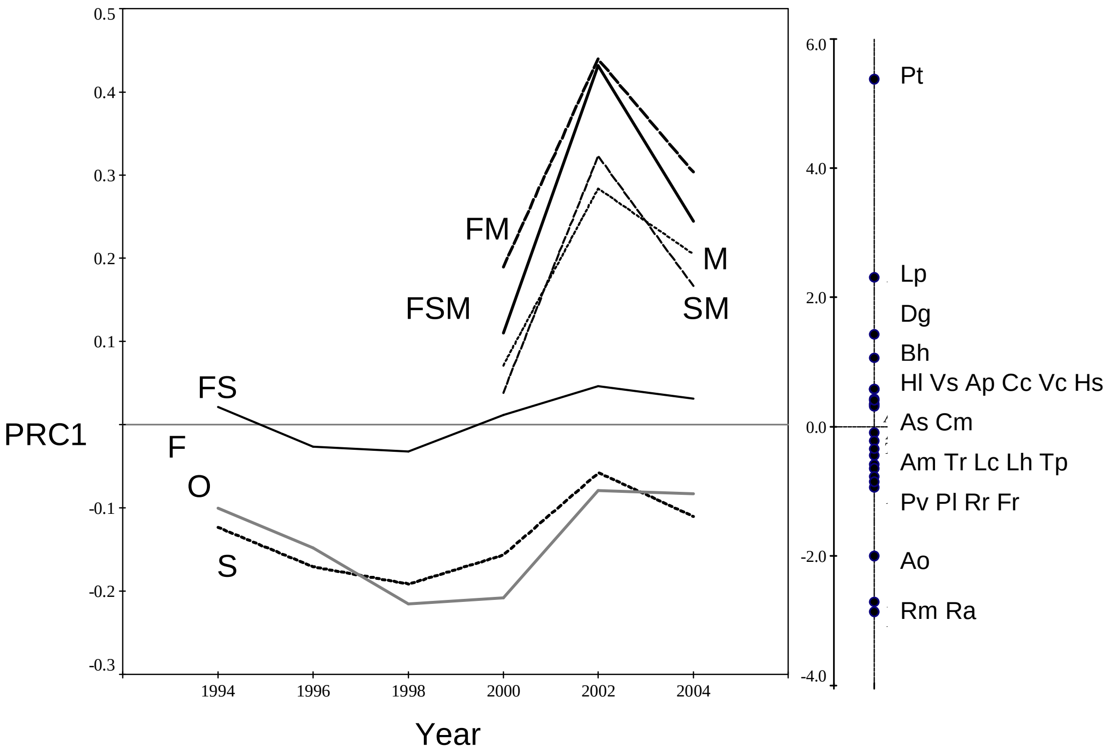
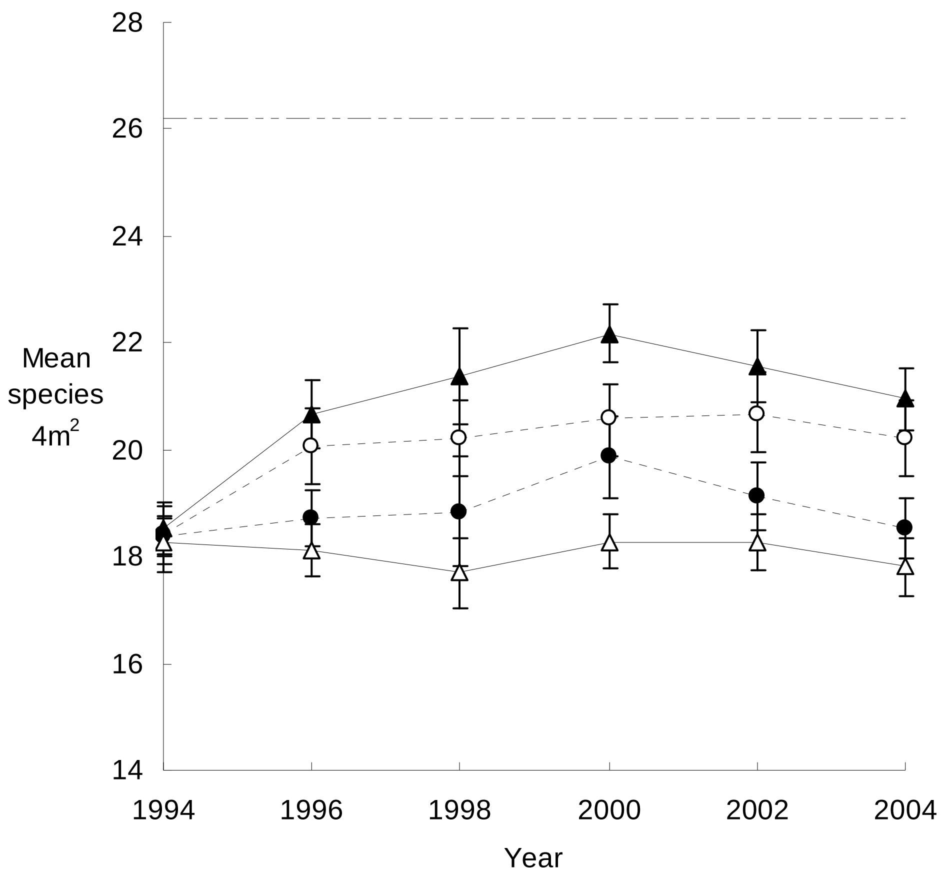


Figure 2



1 Figure 3.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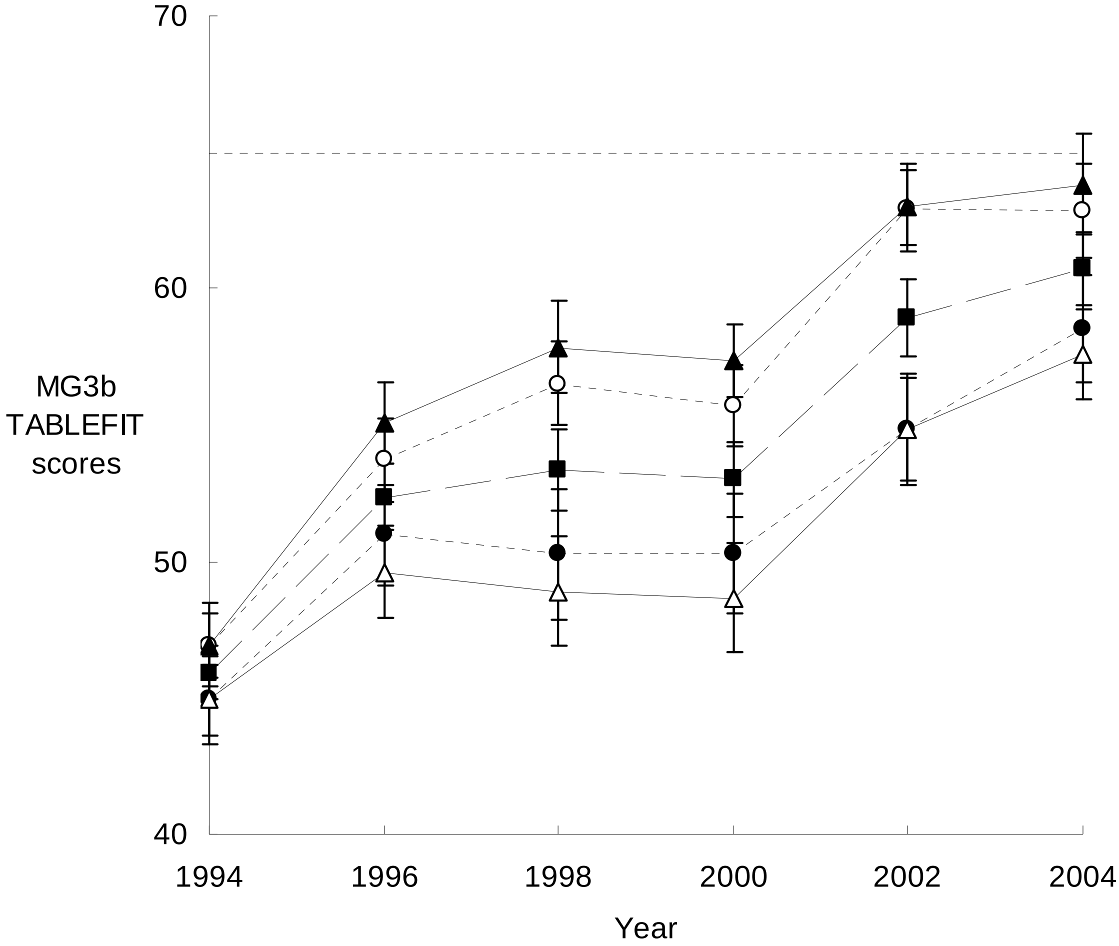
1 Figure 4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MG3b
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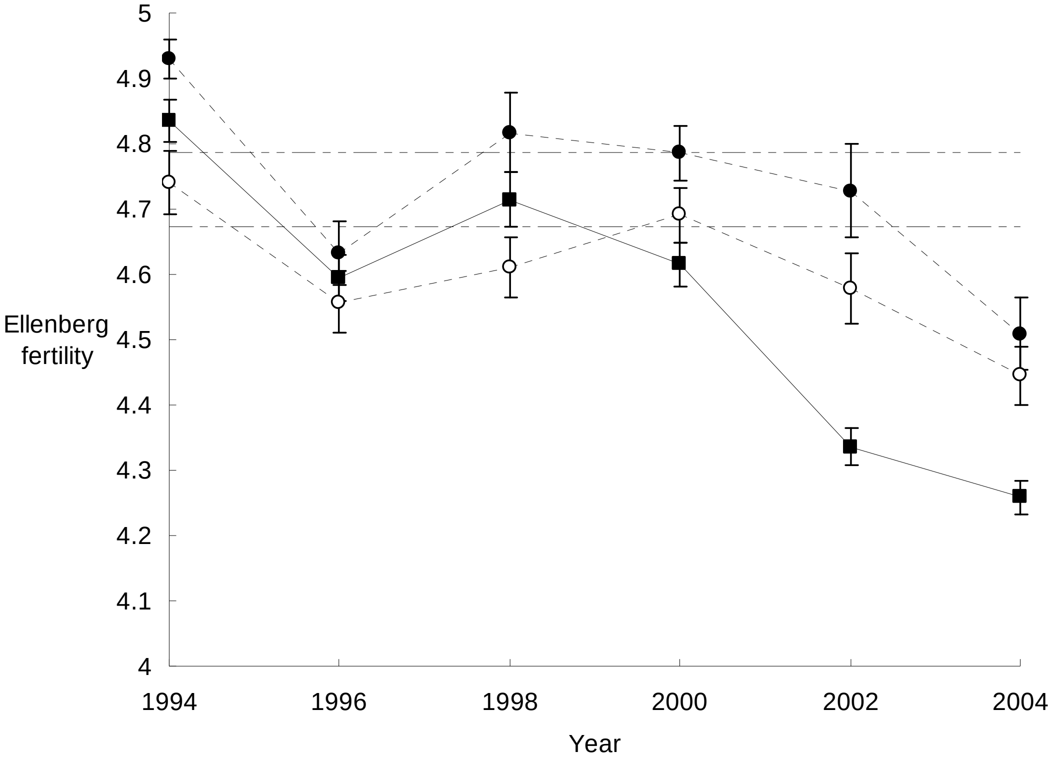
Figure 5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Ellenberg fertility
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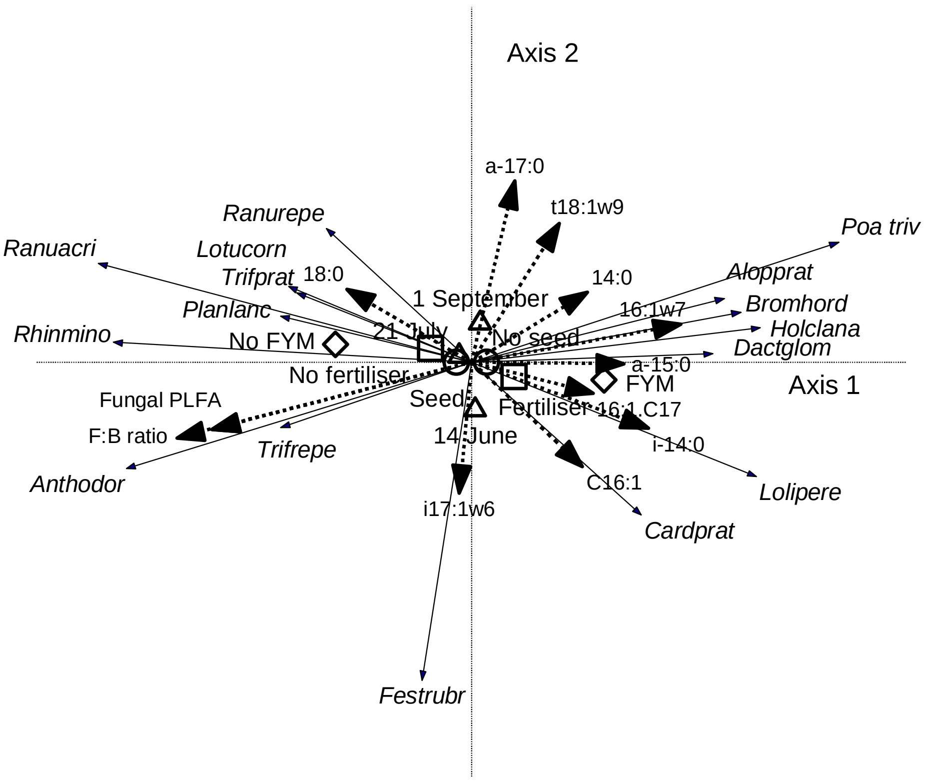
Figure 6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Planlanc
Holclana
Rhinmino
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No FYM
No seed
Dactglom
a-15:0
No fertiliser
FYM
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Anthodor
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Lolipere
i17:1w6
Cardprat
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