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Project identification

1. Defra Project code
   BD1451

2. Project title
   DIVERSIFICATION OF GRASSLAND THROUGH THE MANIPULATION OF PLANT-SOIL INTERACTIONS

3. Contractor organisation(s)
   Lancaster Environment Centre,
   Lancaster University
   Lancaster LA1 4YQ
   United Kingdom

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   £ 667,431 (agreed fixed price)

5. Project: start date... 1st April 2004
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6. It is Defra’s intention to publish this form. Please confirm your agreement to do so.................................YES □ NO □

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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

The overarching goals of this project were: to confirm, across a wide range of grassland types in England, the reported association of high fungal-to-bacterial ratios with agriculturally unimproved, low intensity managed grasslands, relative to moderately intensive, semi-improved and intensively managed agriculturally improved grasslands, to evaluate the potential of these microbial, and other measures, for judging the suitability of sites for re-creation, or enhancement, of botanical diversity, and to identify management options to promote soil fungal-to-bacterial biomass ratios with the aim of enhancing the rate of recovery of botanical diversity in restoration schemes. We designed a range of experiments to test how introductions of particular plant species into swards affect the soil microbial community and in particular the growth of fungi, and the consequences for nutrient cycling and the enhancement of plant diversity. Finally, we set out to provide knowledge that can be used to refine the identification of sites for restoration of botanical diversity, and also provide realistic management options, based on mechanistic understanding of plant-soil associations, for enhancing botanical diversity in grassland.

Results from a national grassland survey revealed that intensive grassland management has led to significant reductions in botanical diversity, species richness and similarity to target unimproved grassland types, and that this was has been associated with increases in soil fertility, measured in terms of increased soil phosphorus (P) and mineral nitrogen (N) content, and reductions in fungal biomass and the fungal-to-bacterial biomass ratio. Collectively, our study indicated a strong association between high fungal-to-bacterial biomass, low input management and high plant species diversity across grassland types representative of the range in environmental conditions and soil types in England. Our study confirmed, therefore, that there is a sound basis for using high fungal-to-bacterial biomass ratios as an indicator of successful grassland biodiversity restoration.

A series of mesocosm and field experiments were carried out to quantify the impact of key plant species on plant-soil feedback, but specifically the development of fungal dominated soils and resultant impacts on vegetation community development. In a mesocosm study we found that individual plant species can strongly modify soil microbial communities and nutrient availability, which then influenced individual plant performance in mixed grassland communities. These
feedback effects were generally independent of soil type or fertility, and were consistently negative in nature. In most cases, individual plant species performed less well in mixed communities planted in soil that had previously supported their own species. These findings suggest that, in addition to soil abiotic factors acting as major drivers of soil microbial communities and nutrient availability, biotic interactions in the form of negative feedbacks induced by the previous presence of individual plant species play a significant role in regulating individual plant performance in mixed grassland communities across a range of soil conditions.

In a cross-site field-based mesocosm experiment, we found that the soil fungal growth was enhanced when key plant species which directly promote soil fungal growth, defined here as ‘direct fungal facilitators,’ were added to species poor grassland communities. The effects of adding *Rhinanthus minor* into grassland communities, defined here as an ‘indirect fungal facilitator’, on soil microbial communities were highly variable across sites and may have been masked by underlying differences in soil properties. For the reporting period, there had been insufficient time for the late successional species, which are characteristic of traditional hay meadows, to become established across treatments and sites. Hence, we were unable to detect feedback effects of changes in soil microbial communities on establishment of late successional species. A two year extension of this study was funded to examine longer-term responses to the experimental treatments, and this will be reported on separately from this report.

At Colt Park, we introduced new experimental treatments to test whether the introduction and removal of certain plant species into existing restoration treatments accelerated vegetation change due to shifts in belowground conditions. We found that the removal of *Rhinanthus minor* did not reduce plant species richness or increase the cover of species indicative of higher fertility, and reseeding with *Trifolium pratense* and *Ranunculus bulbosus* (which did not establish) did not facilitate fungal development or increase F:B ratios within 3 years. However, the application of farmyard manure (FYM) and mineral fertiliser reduced F:B ratios and increased weighted Ellenberg fertility scores of vegetation. Moreover, in a related study, we found that reseeding with the legume *T. pratense*, increased soil C and N storage, especially when these treatments were combined with the cessation of fertiliser. Overall, these data suggest that although the addition of facilitator species and removal of *Rhinanthus minor* had no detectable effect on vegetation and soil microbial communities in the three year period, certain plant species, in this case the legume *T. pratense*, had substantial impacts on other ecosystem services. Further studies are underway to test the generality of this response in a wide range of grasslands.

Another key finding from the Colt Park study was that, over the length of the Colt Park experiment (1996-2008), a strong negative relationship had developed between the F:B PLFA ratio and the Ellenberg fertility index of vegetation across experimental treatments. Plant species composition of the sward has been considerably influenced by the addition of nutrients from fertiliser and FYM over the length of the experiment, with a consequent increase in Ellenberg fertility and decrease in fungal-to-bacterial biomass ratios. However this degree of association between this important soil microbial community characteristic and the Ellenberg fertility has taken 14 years to achieve. This finding provides additional support for the strong coupling of plant and soil microbial communities during restoration management, and a strong link between vegetation restoration and the abundance of fungi relative to bacteria; this, again, points to the potential of this measure to act as an indicator of restoration success.

Mechanistic studies were performed to test whether enhancement of fungal growth and the fungal-to-bacterial ratio in soils, promoted by facilitator species or grassland management, is related to a reduction in soil organic matter quality and a concurrent increase in microbial immobilization of N, which in turns leads to more efficient N supply to plants. Using stable isotope labelling methods across a range of grasslands and in pot experiments, we found that microbial immobilization of N increases strongly with greater fungal biomass, and N leaching decreased with increasing abundance of fungi relative to bacteria, again conforming to our hypothesis that fungal dominated soils immobilize more N in their microbial biomass. We also found that roots took up the largest amount of added N, and significantly more so in species-rich unimproved than in species-poor improved grasslands, suggesting that N transfer to slow-growing plants of species-rich grasslands was more efficient over time in more fungal dominated
soils. Collectively, these findings provide the first direct evidence that high abundance of fungi in soil, which we have shown to be promoted by less intensive grassland management and by certain facilitator species, promotes soil N retention, efficient N supply to plants, and reduces N loss from soil.

Our survey indicates that soil microbial measures, and especially fungal PLFA and the fungal:bacterial PLFA ratio, have strong potential to act as indicators of the conservation value of grassland, in terms of how close the sward and soil conditions are to those of the species-rich target community. Not only are these measures highly sensitive to changes in management intensity but they also explain a relatively large amount of variation in vegetation composition across a broad range of UK grasslands. Based on our data, values of fungal PLFA greater than 1 are associated with extensively managed semi-natural grassland of high conservation value, whereas intensively managed grassland has a mean value of 0.56. For the fungal:bacterial PLFA ratio, extensively managed grasslands of high conservation value have a mean value of 0.08, whereas intensively managed grassland has a mean value of <0.05. On the basis of this analysis, we suggest that values of fungal PLFA of <0.6 and fungal:bacterial PLFA ratio of <0.05 are indicative of grasslands with lesser conservation value. On such soils if species of high conservation value have been introduced, for example as part of an agri-environment agreement, the swards may be relatively unstable until the fungal population has built up. Despite their value as indicators, however, a current weakness of soil microbial community composition measurements is their cost. As an alternative to soil microbial measurements our data indicate that field-level vegetation assessments, and especially the use of the Ellenberg fertility index, could be a cheaper and effective surrogate to be added to a restorability index, especially in calcicolous and mesotrophic grasslands where this measure has considerably more predictive power than any of the measurements of soil microbial, nutrient and carbon (C). We recognise, however, that the Ellenberg score is not without problems: it requires a full species survey including species cover-abundance from quadrat data collected in May-July.

Our final recommendation is that vegetation measures should be supplemented with certain soil measurements, especially given the strong association that we have demonstrated here and in other studies between the plant and soil properties and restoration management. We suggest a range of measures for consideration. On the whole, however, measures of nutrients in soil and vegetation (i.e. N and P) were poorer predictors of vegetation composition than expected, especially in mesotrophic and wet grasslands, and some of these measures (e.g. herbage N and P, and inorganic nutrient availability) are highly variable over time and hence of limited value as indicators of restorability. Our data indicate that soil C might be a useful indicator of grassland restorability, in that it is responsive to changes in management intensity and the larger carbon fractions were consistently among the best predictors of vegetation composition. Moreover, measurements of soil C are relatively cheap and would be of added benefit in terms of gaining information on grassland soil C stocks in the surface soil, and of the potential to promote C stocks via biodiversity restoration management.

Overall, our analysis provides a valuable framework for identifying potential indicators of restorability by assuming that any changes to grass swards and soils in future years will conform to the relationships identified in survey. However, we cannot distinguish between cause and effect without experimental testing of the effects of management treatments on soils of differing fertility. Without experimental evidence, we cannot predict the rate that a given mesotrophic grassland will move over time through the diversity/fertility framework once the main management constraints on diversification have been removed. However, our data provide a strong basis for selecting those measures that are likely to provide the best predictive power in terms of identifying sites that are amenable for restoration management and for tracking their progress, both aboveground and belowground, towards restoration targets.

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**Project Report to Defra**

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information
obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Exchange).

## INTRODUCTION

Restoration schemes aimed at enhancing plant species diversity of improved agricultural grassland have been a key feature of agri-environment policy since the mid 1980’s. Allied to this has been much research aimed at providing policy makers with guidelines on how best to manage grassland to restore botanical diversity. This research includes long-term studies of the consequences for grassland diversity of management treatments such as different hay cut dates, fertilizer additions, seed introductions, and grazing regimes (Pywell et al. 2002; Smith et al. 2003). Studies have also explored the role of introductions of the hemiparasitic plant *Rhinanthus minor* into species-poor swards to debilitate competitive grasses (Pywell et al. 2004; Bardgett et al. 2006). While these studies have been successful in identifying some management features that control plant species diversity in agricultural grassland, they have taken a largely aboveground perspective on plant community dynamics.

This project began at a time of growing recognition that belowground factors, and especially relationships between plant and soil biological communities, strongly interact with management to influence plant species diversity in grasslands (Bardgett 2005). For example, research at that time had shown that species-rich grasslands are associated with a high abundance of fungi relative to bacteria in the soil, with the converse being true of improved grassland (Bardgett and McAlister, 1999; Donnison et al. 2002; Grayston et al. 2001, 2004). Furthermore, it has been suggested that soils with a high fungal component might facilitate the development of less competitive plant species, and that key species, such as legumes and other forbs, may promote fungal growth in soils, thereby creating better conditions for colonisation by less competitive species (Smith et al. 2003). However, it is clear that further research is needed to clarify the role of plant-soil interactions and determine their contribution to the enhancement of botanical diversity of grassland.

The main aim of this study was to confirm, across a wide range of grassland types in England, the reported association of high fungal-to-bacterial ratios with species-rich grasslands relative to improved and semi-improved grasslands, and to use this information to evaluate the potential of these microbial, and other measures, as indicators in agri-environment schemes to target sites for re-creation, or enhancement, of botanical diversity. An additional aim of our study was to identify management options to promote soil fungal-to-bacterial biomass ratios with the aim of enhancing the rate of recovery of botanical diversity in restoration schemes. For this, we designed a range of experiments that enabled us to test how introductions of particular plant species into swards might enhance the growth of fungi, thereby creating soil conditions favourable for enhancement of plant diversity. Finally, a key aim was to provide knowledge that could be used to refine the methods used to identify sites for restoration of botanical diversity, and also provide realistic management options, based on mechanistic understanding of plant-soil associations, for enhancing botanical diversity in grassland. The project included four inter-related objectives:

1. To confirm the reported association of high fungal to bacterial ratios with species-rich grasslands relative to improved and semi-improved grasslands.
2. To quantify the impact of key plant species in the development of fungal dominated soils and resultant impacts on vegetation community development.
3. To identify the mechanisms underlying the influence of plant species on fungal to bacterial ratios in the soil and soil biota influences on vegetation development.
4. To evaluate other potential indicators that could be used in agri-environment schemes to
OBJECTIVE 1: TO CONFIRM THE REPORTED ASSOCIATION OF HIGH FUNGAL TO BACTERIAL RATIOS WITH SPECIES-RICH GRASSLANDS RELATIVE TO IMPROVED AND SEMI-IMPROVED GRASSLANDS.

Background: One of the key aims of BD 1451 was to carry out a comprehensive survey to test how fungal-to-bacterial biomass ratios varied across grasslands of differing botanical diversity and management intensity within a framework of different environmental conditions, including soil types of varying inherent fertility and drainage status, and of different climatic and topographic conditions. The principal aim of this survey was to test the reported association of high fungal to bacterial ratios with species-rich grasslands relative to improved and semi-improved grasslands, an association which had been earlier documented in selected grassland types and within particular regions (e.g. Bardgett and McAlister 1999, Donnison et al. 2002). Here, we provide a summary of the main findings of this survey. A full and detailed account of the results of this survey is provided in Appendix 1.

Approach: We conducted a survey, during June and July of 2005, of 12 geographic regions within England, which covered a broad range of UK grassland communities, including acid, calcicolous, mesotrophic and wet grasslands. Within each region there were five locations, each consisting of three different field types: unimproved (UI) and often designated as Sites of Special Scientific Interest (SSSSI), semi-improved (SI) or improved (I) grassland. These triplets of fields (UI, SI, I) were chosen mainly on the basis of their perceived management intensity; UI fields were identified by being botanically species-rich, and, as far as possible, SI and I fields in a triplet were situated on similar soil types, with similar topography and edaphic characteristics. Within each field, percentage cover of each plant species was recorded from three 1m² quadrats placed at random within a 25m x 25m plot of homogenous vegetation. Within each quadrat, five 2cm diameter 7cm deep soil cores were taken at random and pooled to produce a composite sample for microbial and chemical analysis. Detailed methodology for these methods is provided in Bardgett et al. (2007); but, briefly, the biomass and structure of the soil microbial community was assessed by analysing the ester-linked phospholipid fatty acids (PLFA) composition of the soil, using the method outlined in Bardgett et al. (1996), and air-dried soil was analysed for total carbon (C), total nitrogen (N), total phosphorus (P), Olsen extractable P, extractable potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and pH, using standard methodology (Allen 1989). In addition, the availability of N in fresh soil was measured as: (a) soil concentrations of mineral N (i.e. NH$_4^+$-N and NO$_3^-$-N); (b) net N mineralisation potential; and (c) the concentration of dissolved organic N (DON) in soil (Harrison and Bardgett 2004).

Key findings: The key result from this survey was that we confirmed - at a national scale and across a broad range of grassland types and environmental conditions - the reported high association of fungal-to-bacterial biomass ratios with agriculturally unimproved meadows relative to semi- or improved grasslands, and the strong association of this soil microbial measure with high plant species diversity and low input management; data show that plant species diversity and richness were significantly lower in improved than unimproved grasslands across all regions, and that this was associated with significant increases in soil P and nitrate availability, and herbage N content, and decreases in soil C:N, herbage N:P, fungal biomass and the fungal-to-bacterial biomass ratio (Figure 1). These differences were detected despite large regional differences in most measures, which presumably reflect regional variation in climate, geology and land use history. However, at seven of the twelve regions, we found strong positive and linear relationships between plant species richness and the fungal-to-bacterial ratio, suggesting a possible functional link between belowground properties and plant diversity in grassland. (The absence of relationships in some regions suggested that other factors, such as low soil pH or excessive soil moisture, might act as stronger drivers of plant and soil properties in grassland.) Further confirmation of the strong link between grassland management, vegetation diversity and soil microbial properties emerged from detrended correspondence analysis (DCA) which revealed strong associations between unimproved grassland, high plant species diversity and key soil microbial properties, namely fungal biomass and the ratio of fungal-to-bacterial PLFA (Figure 2). Conversely, improved grassland was associated with high soil fertility, in terms of high soil P, soil nitrate and herbage N, and the abundance of bacteria.
measured by PLFA (Figure 2).

**Figure 1.** Soil and herbage characteristics in unimproved (UI), semi-improved (SI) and improved (I) grasslands. Significant differences are indicated by different letters.

**Figure 2.** Detrended correspondence analysis (DCA) of environmental, soil biological and vegetation characteristics. Circled variables were most correlated with vegetation variation.
The relationship between plant species diversity and weighted Ellenberg fertility, a plant species based measure of soil fertility, varied with vegetation type (Figure 3), but the Ellenberg fertility index always accounted for more variation in plant species diversity than was accounted for by any of the soil measures of fertility, C content or soil microbial community. However, these latter measures were often individually related to plant species diversity, with a strong association being found between high fungal biomass and fungal-to-bacterial biomass ratio and high plant diversity in unimproved meadows. Whilst our data supports the current use of soil nutrient measures, it also provides a sound basis for using high fungal-to-bacterial biomass ratios as an indicator of grassland biodiversity restoration. It is recognised that the associations reported here, being based on a survey rather than a controlled experiment, might not be causal. Also, the selection of unimproved sites, being based primarily on botanical composition, excluded any swards which may have been extensively managed (i.e. receiving little or no fertiliser), yet be botanically poor, perhaps because of occasional use of broad-spectrum herbicides or other management effects.

OBJECTIVE 2: TO QUANTIFY THE IMPACT OF KEY PLANT SPECIES IN THE DEVELOPMENT OF FUNGAL DOMINATED SOILS AND RESULTANT IMPACTS ON VEGETATION.

Background: In recent years, there has been a growing awareness among ecologists of the importance of plant–soil feedback as a driver of plant community dynamics, especially in the context of plant succession and invasion (Bever et al. 1997; Klironomos 2002; Van der Putten 2003), and ecosystem processes such as nitrogen and carbon cycling (Manning et al. 2006; Van der Heijden et al. 2008; Bardgett et al. 2009). By altering the physical, chemical and biological nature of their soil environment, individual plants have the ability to influence their performance relative to their competitors, ultimately leading to changes in plant community composition and diversity. A plant species may influence its associated soil biological community, or other abiotic soil properties, in one of two broad ways, leading to either a positive or negative feedback: a
given plant species may alter its soil environment in a way that increases its own growth rate relative to that of other plant species, resulting in a positive feedback; or, a plant species might alter soil in a way that decreases its own growth rate relative to that of others, resulting in a negative feedback (Bever et al. 1997). Positive plant–soil feedback involves enhanced soil nutrient availability via stimulation of soil microbes involved in mineralization processes or promotion of mycorrhizal fungi that enhance plant nutrient uptake (Klironomos 2002). In contrast, negative feedback involves the accumulation of parasites, pathogens and herbivores of roots (Bever et al. 1997; Klironomos 2002), which can remove carbon and nutrients from plant tissue and reduce root uptake capacity in a species-specific manner, resulting in qualitative differences in plant community composition (Van der Putten 2003; Wardle et al. 2004). Plant–soil feedback describes the net effect of these co-occurring events, namely positive and negative effects, since they are not occurring in isolation.

The aim of this second objective was to quantify the impact of key plant species on plant-soil feedback, but specifically on the development of fungal dominated soils and resultant impacts on vegetation community development. This was done using three different experimental approaches: (1) a mesocosm experiment to test the potential for individual plant species to affect fungal-to-bacterial biomass ratios and soil nutrient availability in a way that feedbacks to the diversity of subsequent plant communities; (2) a larger, cross-site mesocosms experiment designed to test whether the introduction into species-poor grassland of plant species known to promote soil fungal-to-bacterial biomass ratios, termed facilitators, leads to enhanced plant species diversity in grassland communities commonly subject to management for enhancement of botanical diversity; and finally (3) a study based on a long-term field restoration trial of how manipulations of certain plant species affect fungal-to-bacterial ratios and hence vegetation change. A brief description of these experiments and their key findings are given below, whereas a detailed account of each is provided as Appendices 2, 3 and 4.

**Experiment 1: Single species effects and their impact on vegetation dynamics**

**Objectives:** This experiment was done at Lancaster to test how a range of individual plant species, known to be successful in restoration schemes and common in a wide range of NVC communities, affect fungal-to-bacterial biomass ratios and the potential for feedback to the plant community in terms of enhanced botanical diversity and reduced species dominance.

**Approach:** The experiment had two phases: (1) an initial soil conditioning phase of two growing seasons to examine effects of individual plant species on soil microbial properties, and (2) a feedback phase of two years to examine effects of soil conditioning by particular species on botanical diversity of subsequently sown grassland communities. Given that the effects of plant species on microbes are known to vary greatly with soil type and fertility, we used two different soil types, namely a clay loam and sandy alluvial soil, each with two levels of soil fertility, resulting from different long-term management regimes, namely intensively managed fertilized grassland and adjacent unimproved grassland with no known history of artificial fertilizer application. Soil chemical and biological properties were assessed using the same approaches detailed above for objective 1, and a full description of this study, the approaches used, and the results are in Appendix 2 and Harrison and Bardgett (2010).

**Key findings:** A key finding of the first phase of the experiment was that soil abiotic factors, namely soil type and variation in fertility resulting from historic management, were the main determinants of belowground properties. For example, we found that microbial biomass and the abundance of PLFAs synthesized by bacteria and fungi were consistently greater in the clay loam than sandy alluvial soil, whereas the ratio of fungal-to-bacterial phospholipid fatty acids, a measure of the relative abundance of these microbial groups, was greater in sandy than the clay loam soil. These differences reflect the powerful role that inherent differences in soil physical properties of these soils, including texture, pH and moisture status, play as drivers of soil community abundance and structure (Bardgett 2005). Despite this, conditioning effects of different plant species on measures of soil N availability and microbial community structure were detected (Figure 4). Unsurprisingly, the legumes *T. pratense* and *T. repens* increased rates of N mineralization and the availability of inorganic N in soil relative to the grass species, especially *A. odoratum* and *F. rubra*, and also the herb *A. millefolium*. The two legumes also significantly affected microbial community structure, causing a significant reduction in the soil fungal-to-
bacterial PLFA ratio, indicative of a shift towards a bacterial-based energy channel that is typically associated with rapid rates of nutrient cycling (Bardgett et al. 2006; Van der Heijden et al. 2008). The herb A. millefolium also had marked effects on the soil microbial community and nutrient cycling, again demonstrating the potential for individual species to modify the size and structure of the soil microbial community (Figure 4). Collectively, these findings indicate that although plant species can differentially influence soil microbial properties and nutrient cycling, these effects are of less importance than soil abiotic factors in driving these belowground properties.

At the end of the feedback phase, historic management intensity was the most important factor affecting aboveground biomass of mixed communities, being greater in more fertile improved than infertile unimproved soil. Despite this, we found significant plant–soil feedback effects on individual plant growth in mixed communities during the second phase of the study. Moreover, these feedback effects were generally consistent across contrasting soil conditions and were always negative in nature, i.e. individual plant species consistently grew less well in mixed communities planted in soil that had previously supported their own species across both soil

![Figure 4](image)

*Figure 4.* The effect of conditioning soil with nine different plant species on measures of: a) microbial biomass C, b) microbial respiration, c) fungal PLFA and d) fungal:bacterial PLFA biomass ratio. Values are means ± SE. Values with the same letter are not significantly different at the \( P < 0.05 \) level.

types and management regimes (Figure 5). Importantly, we also found that these negative feedback responses, which are most likely due to the accumulation of species-specific pathogens (Klironomos 2002), were predominantly consistent across soils and management histories. These data indicate that while soil microbial communities, nutrient availability and overall plant community biomass are most strongly affected by soil abiotic factors, biotic factors associated with conditioning species and negative feedback play a more important role in
affecting individual plant performance in mixed grassland communities. These findings support the notion that negative feedback is a general phenomenon in mixed species grassland communities, despite the overriding role that soil abiotic factors play in shaping microbial production, nutrient cycling and plant production in temperate grassland.

**Figure 5.** Performance of a) **Anthoxanthum odoratum**, b) **Festuca rubra**, c) **Lolium perenne**, d) **Achillea millefolium**, e) **Plantago lanceolata**, f) **Ranunculus repens**, g) **Lotus corniculatus**, h) **Trifolium pratense** and i) **Trifolium repens** when grown in a mixed community after soil was conditioned by each of the species tested relative to performance in soil conditioned by itself. Values are over both soil types and management histories and are means ± SE. Values marked with a * show significantly different (at the $P < 0.05$ level) performance in away (conditioned by another species) vs. home (conditioned by that species) soil.

**Experiment 2: Impact of key plant species on the development of fungal dominated soils and the resultant impacts on vegetation community development**

**Objectives:** The aim of this study was to test the hypothesis that the introduction of certain plant species into agriculturally species-poor grassland can promote soil fungal growth, relative to that of bacteria, thereby increasing the establishment or late-successional plant species that are key to the successful restoration of species-rich meadows. Specifically, we tested the hypotheses that: (a) direct fungal facilitators (DF) promote soil fungal growth directly, thereby creating soil conditions that are conducive to the establishment and growth of late-successional species and hence the promotion of plant species diversity; (b) indirect fungal facilitators (IF), in this case **Rhinanthus minor**, promotes soil fungal growth indirectly by inhibiting fast-growing plant species associated with enhanced soil bacterial growth (e.g., **Lolium perenne**); and (c) the restoration of plant species diversity within agriculturally species poor grasslands is most rapid when there is a combination of both DF and IF plant species. Moreover, we tested this at three sites, **North Wyke**, Reading and Close House, near Newcastle, with different soil and climatic conditions to test whether responses to plant species additions were context dependent.

**Approach:** Details for the full experimental design are included in Appendix 3, but briefly, at each of the three sites large plastic pots (80cm in diameter and 50cm deep) were buried into the soil.
and vegetation treatments were established in two contrasting soil types taken from permanent grassland, each with two levels of inherent soil fertility resulting from long-term management, namely intensively managed fertilized grassland and adjacent unimproved grassland with no known history of artificial fertilizer application, as in experiment 1. For vegetation, all soils were sown with a mix of six grass species typical of agriculturally improved grassland: *Lolium perenne, Agrostis capillaris, Poa trivialis, Alopecurus pratensis, Holcus lanatus* and *Phleum pratense*. These plants were allowed to establish for one year before applying the remaining two treatments in September 2005, namely the addition or not of a mixture of seven DF species (i.e., *Lotus corniculatus, Prunella vulgaris, Ranunculus acris, R. bulbosus, Anthoxanthum odoratum, Trifolium pratense*, and *Plantago lanceolata* and the addition or not of the IF species *Rhinanthus minor*. With a randomized design of the 4 treatments and 4 replicates, this led to 16 treatments established within 64 plots at each site (Figure 6).

At each of the three sites, the DF and IF plant species were allowed to condition the soils for two years before we added, in September 2007, the same suite of late successional plant species, typical of hay meadow restoration schemes, to each of the 16 different treatments across all three locations: *Briza media, Centaurea nigra Galium verum, Knautia arvensis, Leontodon hispidus, Pimpinella saxifraga, Primula veris, Succisa pratensis*, and *Trisetum flavescens*. Additional late successional species, specific to the different soil types were added at each location, as detailed in Appendix 3. Traditional hay management was applied to the mesocosms throughout the experiment at each site, i.e., simulated grazing (to a height of 5 cm) and trampling in April and August with a July hay cut (to a height of 5 cm). Vegetation and soil were sampled annually between 2004–2008, and soils were sampled in 2005, 2006 and 2008 for soil chemical and microbial analysis, using PLFA.

**Figure 6**: a) Layout of the 64 mesocosms at North Wyke; b) grass seedlings in the North Wyke intensive clay soils November 2004; and c) North Wyke unimproved alluvial soils following the simulated April “sheep” grazing.
**Key findings:** Across all sites, the addition of DF species reduced biomass of the original grass species, and treatments where *R. minor* was present were characterized by a lower total hay yield, reduced grass biomass of the original six grasses, and greater soil inorganic N availability, measured as the nitrate and total inorganic N, than treatments where the IF was absent. As hypothesised, adding DF species into species poor grassland also enhanced soil fungal growth across all three sites by 2008, although this trend was not significant at the Reading site where the increase was only detected in chalk soil (Figure 7a-c). The IF treatment with *Rhinanthus minor* had a less consistent effect on soil fungal growth across sites, which most likely reflects underlying differences in soil properties across sites and the temporal nature of this species, being an annual, and hence its influence on soil. Across all three years and sites there was also a trend for F:B PLFA ratio to be greater where DF were sown, yet this was only significant at North Wyke. At Reading, there was a non-significant trend for the F:B to be greater where *R. minor* was sown on chalk soils, whilst on the neutral soils this measure was slightly greater where *R. minor* was absent. For the other two sites, the trend, albeit not significant, was for F:B to be lower where the IF had been sown. In terms of soil nutrient availability, there was a trend for inorganic N availability to be greater where the IF has been sown at all three locations (Fig 6d-f), though the result is only significant at Reading and Close House in 2008, and likewise, soil nitrate concentrations were greater where *R. minor* was present, though this was again only significant at Reading and Close House. For the reporting period, there was insufficient time for the late successional species, which are characteristic of traditional hay meadows, to become established. Therefore, the experiment received funding to be continued until 2012 to provide more data to predict the conditions under which their establishment is most successful. These results will be reported separately.

![Figure 7](image)

**Figure 7.** Effects of DF introduction on fungal biomass in 2008, measured by PLFA, at Reading (a), Close House (b) and North Wyke (c). Impact of IF on soil inorganic N in 2008 at Reading (d), Close House (e) and North Wyke (f).

Overall, the results of this study show that the introduction of key plant species into species-poor grassland have the capacity to directly promote soil fungal growth across a wide range of climatic and soil conditions. The consequences of this for the establishment of desired haymeadow plants sown into the sward are as yet unknown, due to the short timescale of the experiment. However, we predict that the promotion of fungal rather than bacterial dominated soils by DF species will create soil conditions that are more suitable for less competitive plant species, enabling them to gain a hold in the sward.
Experiment 3: Colt Park field trial

Objectives: The aim of this part of the project was to examine the long-term facilitation of soil fungi under conditions typical of agri-environment scheme agreements by utilising the developing species and soil trends within the long-term Colt Park Field Experiment, in the Yorkshire Dales (Smith et al. 2003, 2008). The design of this experiment enabled a new treatment to be imposed whilst maintaining the original treatments in reduced plots. Here, changes in fungal-to-bacterial ratios have been associated with introduced legumes and hay rattle after a long lag phase. Here, we tested the hypothesis that continued vegetation development will be conditioned by changed soil conditions induced by plant species sown in 1990-92, and that change will be accelerated by (1) Rhinanthus minor, which is known to debilitate the growth of Lolium perenne, a species known to lower the fungal-to-bacterial biomass ratio; and (2) A ‘fungal facilitator’ treatment with the addition of R. minor seed to supplement the existing population, and with seed of Trifolium pratense.

Approach: A full description of the experimental design at Colt Park is given in Appendix 4, but, briefly, we introduced a new phase of management regimes in 2004-2008 with two changes to the experimental design. The first was three new reseed treatments to replace the phase 1 cutting date treatments, which had not been applied since 1998. These reseed treatments were: (1) no-reseed; (2) reseed with Trifolium pratense; and (3) reseed with Trifolium pratense plus Ranunculus bulbosus. Each of these was applied to one randomly selected example of each of the original cut date treatments, to ensure that any residual long-term effects of these cut dates did not confound the reseed treatments. We also subdivided each of the plots and in early-June each year removed Rhinanthus minor from one of the subplots.

Key findings: We found that the removal of Rhinanthus minor from 2005 did not reduce plant species richness or increase the cover of species indicative of higher fertility. We suggest that this was a consequence of an unexpected decline in populations of R. minor across the whole trial, including that which had been expected to persist in the unweeded plots. Also, reseeding with Trifolium pratense and Ranunculus bulbosus (which did not establish) did not facilitate fungal development or increase F:B ratios within 3 years. However the application of farmyard manure (FYM) and mineral fertiliser reduced F:B ratios and increased weighted Ellenberg fertility scores of vegetation. Moreover, in a related study, we found that reseeding with the legume Trifolium pratense, increased soil C and N storage by 2006, especially when these treatments were combined with the cessation of fertiliser (De Deyn et al. 2011). These high rates of C and N accumulation were associated with reduced ecosystem respiration, increased soil organic matter content and improved soil structure. Overall, these data suggest that although the addition of facilitator species and removal of Rhinanthus minor produced no detectable effects on vegetation and soil microbial communities in the three year period, certain plant species, in this case the legume Trifolium pratense, had substantial and unexpected impacts on other ecosystem services, in this case promoting soil carbon sequestration.

Another key finding was that, over the length of the Colt Park experiment (1996-2008), a strong negative relationship developed between the F:B PLFA ratio and the Ellenberg fertility index of vegetation across experimental treatments (Figure 8). As reported in Appendix 4, the species composition of the sward has been considerably influenced by the addition of nutrients from fertiliser and FYM over the length of the experiment, with a consequent increase in Ellenberg fertility and decrease in fungal-to-bacterial biomass ratios. However this degree of association between this important soil microbial community characteristic and the Ellenberg fertility has taken 14 years to achieve. There were no significant relationships in the early years of the trial, in 1996 when fertiliser was applied, or in 2000, after the start of FYM applications in 1998 (Figure 8). In 2008 the F:B ratio decreased rapidly with increasing Ellenberg fertility (R²=0.346, F=74.6, P<0.0001) (Figure 8).
OBJECTIVE 3: IDENTIFY THE MECHANISMS UNDERLYING THE INFLUENCE OF PLANT SPECIES ON FUNGAL-TO- BACTERIAL RATIOS IN THE SOIL

Background: The experiments done in objectives 1 and 2 have advanced understanding about the way that individual plants impact on fungal-to-bacterial biomass ratios, and how such changes relate to grassland management, soil chemical properties and vegetation dynamics. In this objective, we used stable-isotope approaches to test how elevated fungal-to-bacterial biomass ratios influence N cycling in grassland soil, specifically testing the idea that enhancement of fungal growth and the fungal-to-bacterial ratio in soils, promoted for instance by facilitator species or grassland management, is related to a reduction in soil organic matter quality and a concurrent increase in microbial immobilization of N, which in turns leads to more efficient N supply to plants. We predict that this is due to high levels of microbial immobilisation, or retention, of N in fungal dominated soils and subsequent transfer of this microbial N to plants, enhancing their competitive ability relative to faster growing species that require access to inorganic N.

Approach: The above was tested through a combination of field and pot experiments, involving $^{15}$N-labeling into intact soils, and based on grasslands of contrasting management intensity, as used in Objective 2. Grasslands of contrasting management intensity were selected, based on the findings of objective 1 where we showed that agriculturally improved grasslands are characterized by bacterial-dominated microbial communities, whereas unimproved, extensively managed grasslands are characterized by more fungal-dominated microbial communities. Intact soil columns were labelled with $^{15}$NH$_4$$^{15}$NO$_3$ solution (99.5% enriched), and columns were leached and destructively harvested 48 hours and two months after $^{15}$N addition and the fate of added $^{15}$N in leachate, plant tissue (shoot and root), microbial biomass, and soil pools was measured, using methods of Bardgett et al. (2003).

Key findings: Consistent with our prediction, we found that soil inorganic N availability in the field decreased with increasing abundance of fungi relative to bacteria. Likewise, less inorganic N leached from intact soil columns from species rich unimproved grasslands than from species-poor improved grasslands, and total N leached from columns in the greenhouse was strongly related to total N leaching in the field. In the labelling experiment we found that microbial immobilization of added $^{15}$N increased strongly with greater fungal biomass, and $^{15}$N leaching decreased with increasing abundance of fungi relative to bacteria, again confirming our hypothesis that fungal dominated soil immobilize more N in their microbial biomass (Figure 9). The next question was whether this enhanced immobilization of N in fungal dominated soils led...
to more efficient N transfer to plants. We found that roots took up the largest amount of added \(^{15}N\), and significantly more so in columns from unimproved than improved grasslands (Figure 10), suggesting that N transfer to slow-growing plants of species-rich grasslands was more efficient over time in more fungal dominated soils, thereby again confirming our hypothesis. In contrast, shoot uptake did not differ between the two management intensities and increased towards the end of the experiment (Figure 10). Taken together, the amounts of added \(^{15}N\) retained in microbial, soil, and above- and belowground vegetation pools were greatest in unimproved grasslands, and the retention of added \(^{15}N\) increased with greater fungal biomass. Collectively, these findings provide the first direct evidence that high abundance of fungi in soil, which we have shown to be promoted by less intensive grassland management and by certain facilitator species, promotes soil N retention, efficient N supply to plants, and reduces N loss from soil.

![Figure 9](image)

**Figure 9.** \(^{15}N\) leaching and microbial \(^{15}N\) immobilization in the glasshouse experiment. A, \(^{15}N\) leaching in the glasshouse experiment as explained by F/B ratio. Sampling date P = 0.0006, F/B ratio P = 0.0003, Sampling date x F/B ratio P < 0.0001, \(R^2 = 0.72\). B, Microbial \(^{15}N\) uptake as explained by fungal PLFA. Sampling date P = 0.03, Fungal PLFA P < 0.0001, Sampling date x Fungal PLFA P = 0.0001, \(R^2 = 0.58\). Filled symbols represent improved grasslands, open symbols unimproved grasslands; diamonds represent 48-hour-sampling, triangles two-month-sampling. Solid lines are the predicted relationship for 48-hour-sampling, dashed lines are predicted relationships for two-month-sampling.

**OBJECTIVE 4. TO EVALUATE OTHER POTENTIAL INDICATORS THAT COULD BE USED IN AGRI-ENVIRONMENT SCHEMES TO TARGET SITES FOR RE-CREATION OR ENHANCEMENT OF GRASSLAND.**

**Background:** The principal goal of Objective 4 was to provide a reliable and cost-effective toolbox of indicators of grassland restorability based on the results obtained from the Objective 1 sampling framework, the experiments conducted in Objectives 2 and 3, and a literature review and data from a range of existing restoration/reversion experiments. A literature review was completed to meet Objective 4.1 (Appendix 5), but the principal aim of this Objective was to provide a practical, simple to use ‘restorability index’ for assessing the potential of a grassland site for colonization by new species to raise the diversity/species richness to levels associated with ‘traditional’ low intensity management. This index was also required for confident assessment of the progress of diversity restoration of grassland in an agri-environment scheme. We define ‘restorability’ as the ability of a soil-vegetation complex to enable colonization by new species to raise plant species richness and diversity to levels associated with traditionally managed, low input grasslands.

**Approach:** We used the DIGFOR survey of grassland soil and vegetation characteristics across different grassland types and regions in England to provide a basis for a restorability index: the potential of a site to change to high nature conservation value in terms of semi-natural plant diversity. Specifically we interrogate the data gained from this survey to address two questions:
Figure 10. $^{15}$N pools in improved (black bars) vs. unimproved grasslands, 48 hours and two months after $^{15}$N addition. A, $^{15}$N leached (Sampling date $F_{1,42} = 58.1, P < 0.0001$); B, $^{15}$N uptake in microbial biomass (Management $F_{1,40} = 7.5, P = 0.003$, Sampling date $F_{1,40} = 9.7, P = 0.009$, Management x Sampling date $F_{1,40} = 5.2, P = 0.03$); C, $^{15}$N in roots (Management $F_{1,42} = 6.9, P = 0.01$, Sampling date $F_{1,42} = 3.1, P = 0.08$, Management x Sampling date $F_{1,42} = 0.03, P = 0.85$); D, $^{15}$N in aboveground plant biomass (Management $F_{1,42} = 0.06, P = 0.80$, Sampling date $F_{1,42} = 59.6, P < 0.0001$, Management x Sampling date $F_{1,42} = 0.03, P = 0.87$). E, amount of $^{15}$N retained in the different pools in improved vs. unimproved grasslands, after 48 hours and two months (Management $F_{1,40} = 5.7, P = 0.02$, Sampling date $F_{1,40} = 0.2, P = 0.69$, Management x Sampling date $F_{1,40} = 0.005, P = 0.94$). Bars represent means (n = 12) ± 1SE.

(1) are there good soil indicators, including measures of soil microbial communities and nutrients, of the positioning of grasslands along the semi-natural plant species richness – management intensity gradient which could provide a basis for identifying grassland sites suitable for restoration; and (2) are there good, low cost vegetation and soil indicators that can act as surrogates for more costly and time consuming measures of the soil microbial
community? As mentioned, the data used for this analysis were based on measures of a range of soil properties and vegetation characteristics along gradients of management intensity - from very high, as typified by agriculturally improved productive grassland, to extensively managed, high nature conservation value grassland. These data were obtained from a range of sites covering all of the major grassland types and regions in England. A basic assumption of the analysis was that the soil and vegetation characteristics at the extensive end of the management intensity gradient represented restoration targets in terms of plant diversity and similarity of the sward to the target unimproved grassland. Indices of restorability and the magnitude of their differences along the management intensity gradient would, therefore, be identified.

**Key findings:** A detailed account of the toolbox and its potential application are given in Appendix 5. Our survey indicates that soil microbial measures, and especially fungal PLFA and the fungal:bacterial PLFA ratio, have strong potential to act as indicators of the conservation value of semi-natural grassland, in terms of how close the sward and soil conditions are to those of the species-rich target community. Not only are these measures highly sensitive to changes in management intensity, but they also explain a relatively large amount of variation in vegetation composition across a broad range of UK grasslands. Based on our data, values of fungal PLFA greater than 1 are associated with extensively managed semi-natural grassland of high conservation value, whereas intensively managed grassland has a mean value of 0.56. For the fungal:bacterial PLFA ratio, traditionally managed grasslands of high conservation value have a mean value of 0.08, whereas intensively managed grassland have a mean value of <0.05. On the basis of this analysis, we suggest that values of fungal PLFA of <0.6 and fungal:bacterial PLFA ratio <0.05 are indicative of grasslands with lesser conservation value. On such soils, if species of high conservation value have been introduced, for example as part of an agri-environment agreement, the swards may be relatively unstable until the fungal population has built up. Despite their value as indicators, a current weakness of soil microbial community composition measurements is their cost. As an alternative to soil microbial measurements our data indicate that field-level vegetation assessments, and especially the use of the Ellenberg fertility index, could be a cheaper and effective surrogate to be added to a restorability index, especially in calcicolous and mesotrophic grasslands where this measure has considerable predictive power (Figure 3), and much more than any soil-based measure can offer. We recognise, however, that the Ellenberg score is not without problems: it requires a full species survey including species cover-abundance from quadrat data collected in May-July.

Our final recommendation is that vegetation measures should be supplemented with certain soil measurements, especially given the strong association that we have demonstrated here and in other studies between the plant and soil properties and restoration management. We suggest a range of measures for consideration. On the whole, however, measures of nutrients in soil and vegetation (i.e. N and P) were relatively poor predictors of vegetation composition, especially in mesotrophic and wet grasslands, and some of these measures (e.g. herbage N and P, and inorganic nutrient availability) are highly variable over time and hence of limited value as indicators of restorability. Our data indicate that soil C might be a useful indicator of grassland restorability, in that it is responsive to changes in management intensity and the larger carbon fractions were consistently among the best predictors of vegetation composition. Moreover, measurements of soil C are relatively cheap and would be of added benefit in terms of gaining information on grassland soil C stocks in the surface soil, and of the potential to promote C stocks via biodiversity restoration management.

Overall, our analysis provides a valuable framework for identifying potential indicators of restorability by assuming that any changes to grass swards and soils in future years will conform to the relationships identified in survey. However, we cannot distinguish between cause and effect without experimental testing of the effects of management treatments on soils of differing fertility. Without experimental evidence, we cannot predict the rate that a given mesotrophic grassland will move over time through the diversity/fertility framework once the main management constraints on diversification have been removed. However, our data provide a strong basis for selecting those measures that are likely to provide the best predictive power in terms of identifying sites that are amenable for restoration management and for tracking their progress, both aboveground and belowground, towards restoration targets.
OVERALL DISCUSSION
The overarching goal of this project was to confirm, across a wide range of grassland types in England, the reported association of high fungal-to-bacterial ratios with agriculturally unimproved semi-natural grasslands relative to improved and semi-improved grasslands, and to use this information to evaluate the potential of these microbial, and other measures, as indicators in agri-environment schemes to target sites for re-creation, or enhancement, of botanical diversity. We also set out to provide a mechanistic basis for identifying management options to promote soil fungal-to-bacterial biomass ratios with the aim of enhancing the rate of recovery of botanical diversity in restoration schemes, and to provide knowledge that can be used to refine the identification of sites for restoration of botanical diversity based on mechanistic understanding of plant-soil associations, for enhancing botanical diversity in grassland.

The results reported here of the first five years of this project, have advanced our understanding of the role of plant-soil interactions in grassland restoration in several ways, and have also provided a possible basis for identifying sites that are suitable for restoration management. Perhaps one of the most striking findings comes from our field survey, which revealed that, across a wide range of grasslands, soil conditions, and climatic conditions, there is a strong and consistent association between high fungal-to-bacterial biomass, low input management and plant species diversity in grassland. Not only does this finding confirm the suggestions of previous studies, done at isolated locations and limited numbers of sites (Bardgett and McAllister, 1999; Donnison et al. 2002; Grayston et al. 2001, 2004), but they also provide a sound basis for using fungal-to-bacterial biomass ratios as a key indicator of the success of grassland biodiversity restoration, which was an additional aim of our study. In fact, another key finding of our survey was that soil microbial measures, and especially fungal PLFA and the fungal:bacterial PLFA ratio, have strong potential to act as indicators of the conservation value of semi-natural grassland, in terms of how close the sward and soil conditions are to those of the species-rich target community. Not only are these measures highly sensitive to changes in management intensity, as shown here, but we also found them to explain a relatively large amount of variation in vegetation composition across our sampled grasslands. Specifically, we found that values of fungal PLFA greater than 1 are associated with traditionally managed semi-natural grassland of high conservation value, whereas intensively managed grassland has a mean value of 0.56. For the fungal:bacterial PLFA ratio, traditionally managed grasslands of high conservation value have a mean value of 0.08, whereas intensively managed grasslands have a mean value of <0.05. On the basis of this analysis, we suggest that values of fungal PLFA of <0.6 and fungal:bacterial PLFA ratio <0.05 are indicative of grasslands with lesser conservation value. On such soils, if species of high conservation value have been introduced, for example as part of an agri-environment agreement, the swards may be relatively unstable until the fungal population has built up. Despite their value as indicators, we recognise that a current weakness of soil microbial community composition measurements is their cost. As a result, we recommend, based on our findings, that an alternative to soil microbial measurements are field-level vegetation assessments of the Ellenberg fertility index, which could be a cheaper and effective surrogate to be added to a restorability index, especially in calcicolicus and mesotrophic grasslands where this measure has considerable predictive power. We recognise, however, that the Ellenberg score is not without problems: it requires a full species survey including species cover-abundance from quadrat data collected in May-July.

A key aim of this project was to identify the mechanisms by which plants modify soils and their microbial communities and determine how such changes influence vegetation development. The general idea here is that such understanding might enable plant communities to be managed in a way that enhances the development of fungal dominated soils, which in turn will facilitate more efficient nutrient cycling and restoration of vegetation diversity. We performed a series of experiments which provide significant insights into this issue, and point to the potential for plant communities to be manipulated in a way that might enhance ecosystem services, especially efficient N cycling and carbon storage in soil. Specifically, our studies, which have been in a range of experimental systems, reveal four key findings. First, we have shown that individual plant species can strongly modify soil microbial communities and nutrient availability, and that such changes can feedback to influence individual plant performance in mixed grassland communities, therefore potentially affecting vegetation dynamics. Second, we have shown that soil fungal growth in soil can be enhanced when key plant species, defined here as ‘direct fungal facilitators,’ are added to species poor grassland communities, and that this response occurs
across a wide range of soils and under different climatic conditions. Third, we used stable isotope labelling methods to show that microbial immobilization of N increases strongly with greater fungal biomass, and N leaching decreases with increasing abundance of fungi relative to bacteria, again confirming our hypothesis that fungal dominated soils immobilize more N in their microbial biomass. We also found that plants took up the largest amount of added N, and significantly more so in species-rich unimproved than species-poor improved grasslands, suggesting that N transfer to slow-growing plants of species-rich grasslands was more efficient over time in more fungal dominated soils. Finally, we have shown in the field that the long-term development of plant communities under restoration management is tightly coupled with the soil microbial community, especially the fungal-to-bacterial PLFA ratio, and that, over time, becomes negatively related to the Ellenberg fertility index. This finding provides additional support for a strong link between vegetation restoration and the abundance of fungi relative to bacteria; this, again, points to the potential of this measure to act as an indicator of restoration. Also, while the addition of key plant species to restoration treatments had no impact on microbial communities over the three-year period studied, we did find significant impacts of on soil carbon storage, which increased significantly with the addition of *Trifolium pratense* (De Deyn et al. 2011). Although these studies collectively provide evidence for strong coupling of plant and soil microbial communities during restoration management, it is important to note that we are still scratching the surface in terms of our understanding of how plant species and changes in plant communities impact on soil communities, in that many, as yet unidentified, mechanisms are involved (Bardgett and Wardle 2010). However, what they do point to is that a mechanistic understanding of vegetation restoration requires a combined aboveground-belowground approach, and that management for vegetation diversity and other ecosystem services, such as soil carbon storage and nutrient retention, requires consideration of what goes on belowground.

Overall, our analysis provides a valuable framework for identifying potential indicators of restorability, especially the Ellenberg fertility and the fungal-to-bacterial biomass ratio. Our results also shed light on the mechanisms by which plant and soil communities might be manipulated to encourage fungal dominated soils that can reap benefits for restoration of biodiversity and ecosystem services, including carbon and nitrogen retention (De Deyn et al. 2011; De Vries et al. 2012). However, many questions remain, especially concerning the mechanisms and timeframes involved in encouraging fungal dominated soils in grassland, and the best routes for ensuring that management aimed at encouraging plant-soil interactions that promote plant diversity and ecosystem services are compatible with goals of economic yield.

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**REFERENCES**


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References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.


