The role of farmyard manure in the maintenance of botanical diversity in the traditionally managed hay meadows of the Pennines.

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Abstract

Traditionally managed hay meadows of the Pennines are highly prized for their botanical diversity. In recent decades there has been a drastic reduction in these meadows because of the negative effects on botanical diversity of widespread management changes, such as the application of inorganic fertiliser and the switch from hay to silage production. The consequences of changes in grazing regime, cut date and inorganic fertiliser application have been well documented. However, although farmyard manure application is an integral part of long term meadow management it has as yet received less scientific attention.

The application of farmyard manure provides a possible mechanism by which seed contained within hay cut from the meadows can subsequently be returned to the meadow. The dispersal of seed and establishment of plants within traditionally managed hay meadows is an important area of study because of the problems associated with attempts to re-create meadows in the Pennines and throughout the UK, an important goal of agri-environment schemes.

Field, glasshouse and laboratory experiments are described which attempt to establish the viable seed content in samples of hay from botanically diverse meadows and how subsequent feeding to livestock affects the viable seed content of the farmyard manure produced. The experiments also explored the extent to which seed remained viable during the time that manure was stored prior to spreading, thus affecting its capacity to germinate once spread or to become incorporated into the soil seed bank. The spreading of farmyard manure has often been observed to create bare patches within the meadow vegetation. It was therefore hypothesised that these patches may be suitable niches for the germination of seeds from within the manure itself or from the soil seed bank, and this was investigated using field experimentation.

Comparison of the original vegetation with the viable seed content of hay, manure and dung from cattle fed exclusively on meadow hay from two traditionally managed sources produced some interesting results. The meadow vegetation contained a range of prominent perennial herbs such as *Geranium sylvaticum,*

Sanguisorba officinalis and *Filipendula ulmaria* which are considered to be important from a conservation management viewpoint. However, germination of the seed within the hay cut from these meadows failed to provide any evidence for the occurrence of such species within the hay. Either the typical mid July hay cut comes before these species produce ripe seed or the seed was lost from the hay during the turning and baling operations. Viable seeds of the early flowering annual herb *Rhinanthus minor*, which is also considered by ecologists to be desirable, were also absent from the hay. The most abundant species in the hay were generally grasses, with *Poa trivialis* especially prominent, and less important, often annual, herb species.

The feeding of this hay to cattle and subsequent germination of seed within the dung showed that the digestive system of the cattle was extremely damaging to seed viability. Proportionally *P. trivialis* became even more dominant within the dung samples with many of the less common species in the hay failing to have any viable seed within the dung. In contrast to the dung, the fresh manure collected from the two farms contained a larger quantity of seed, and more species than the dung. These samples were in fact more comparable to the hay samples in terms of species composition although not quantity. This suggests that the majority of the viable seed which is incorporated into manure does not pass through the digestive tract of the livestock, but rather falls directly from feed racks onto the barn floor.

Confirmation of the negative effect of the digestive processes of cattle on seed viability was achieved in a laboratory experiment. A three stage process was used to mimic the effects of chewing, rumen digestion and post ruminal digestion. Five species were tested; two grasses *P. trivialis* and *Anthoxanthum odoratum* which occurred within the hay samples, the annual herb *Myosotis arvensis* which was found within hay samples and two perennial herbs *F. ulmaria* and *S. officinalis* which were absent from the hay samples. Whilst the effects of chewing and ruminal digestion varied between the five species tested the post ruminal digestion was extremely damaging in all five species. These results suggested that the prominence of *P. trivialis* in dung samples was due more to the high quantity of seed within the hay rather than to any increased ability to survive digestion. The perennial herbs, *F. ulmaria* and *S. officinalis* were able to survive digestion at least as well as *P.*

trivialis and so given appropriate management of hay cut timing could become incorporated into manure for subsequent dispersal via this route.

By comparing the viable seed content of fresh manure with that in samples stored for 3 months, 6 months and 12 months it was clear that the seed content of the manure did not reduce until it had been stored for a period longer than 6 months. Once again those species with the greatest quantity of seed in fresh manure were the ones which were able to survive within the older manure samples. The burial in manure of known quantities of the same seeds as used in the laboratory digestion experiment confirmed this result, and also showed that herb species absent from the hay and manure are at least as capable of survival within manure heaps as species such as *P. trivialis* which dominated the manure samples collected from farms.

In order to assess the role that manure application to meadows may have in supplying seed to the soil seed bank, soil cores were extracted from the meadows at the two farms used in the study and the seed content estimated by seed germination. The soil seed bank contained all of the species found within the manure samples as well as a range of earlier flowering species including the annual herb *R. minor*. Again the longer lived perennial herb species valued from a bio-diversity perspective were largely absent from the soil seed bank or only present in very low quantities. The species make up of the seed bank within these diverse hay meadow communities was found to be more akin to the species make up of species poor pasture communities. This suggests that seed set by many of the ecologically desirable species is not a regular occurrence within Pennine meadows. The large quantity of seed of certain species found within manure applied to the meadows means that it could play a significant role in the build up of seed within the soil seed bank, although these species may be expected to be recruited to the soil seed bank via other routes such as seed rain during the period of crop growth and the hay making operations.

Field experimentation with observation of the colonisation of gaps created by farmyard manure application showed that vegetation colonising these gaps did not compare to the seed content of the manure or the soil seed bank, but rather the surrounding vegetation. Colonisation of these gaps did not give rise to vegetation containing a higher quantity of species indicative of the manure or soil seed content. Perennial herb species absent from both the manure and the soil seed bank were amongst the colonisers of the these gaps suggesting that vegetative re-growth is an important process in the maintenance of long term botanical diversity within such meadows. Whilst it remains possible that during years when the hay cut is later than usual, seed production and subsequent incorporation into the hay of more desirable later flowering herbs could occur, the overwhelming weight of evidence suggests that the farmyard manure from cattle fed meadow hay is not a significant contributor to the dispersal of ecologically valuable components of meadow vegetation. Indeed, the results suggest that seed production may be less important than the vegetative propagation of many species.

Table of Contents.

1. Introduction

1.1 Background

Traditionally farm management of grassland has been split into two main types, meadows and pastures (Rackham 1986). Traditional hay meadow plant communities are semi-natural grasslands that have been opened from woodland, or reclaimed from wetland sites. Consistently managed for centuries, they provide fodder for over wintering livestock. Hay meadows can be distinguished from pastures by the removal of grazing stock during the spring-summer period whilst plant growth is at an optimum. The resulting crop is then cut and dried in the field. Evidence for haymaking has been found in Roman deposits but the practise could conceivably predate this period (Greig 1988).

The spring 'shut-up' of meadows and the subsequent growth of taller vegetation has allowed distinct floristic differences to develop between neighbouring pastures and meadows (Rodwell 1992). Tansley (1939) found lowland meadows of England to contain a higher proportion of dicotyledonous herbs and more robust grasses whilst adjacent pastures were restricted to fine leaved grasses and low growing herbs. These meadows were known to have been managed as hay meadows for centuries resulting in the plant communities being comparatively stable. Such communities contain plants which are relatively intolerant of grazing. Plants such as *Fritillaria meleagris* and *Colchicum autumnale* in the south and east of England and *Geranium sylvaticum* and *Trollius europeus* in the north and west (Hopkins 1990).

The term 'traditional' is often used with reference to the management of farms and meadows. Its meaning is however vague and is often confused with neglect. The main features relating to the grassland include a lack of ploughing, the use of manures from the farm livestock and consistent management over long periods of time (Hopkins 1990).

Before the widespread agricultural improvements which took place after the Second World War (Hopkins and Hopkins 1994) hay meadows were distinctive and common features of the English landscape. Indeed before the development of tractors and motor vehicles hay meadows also provided 'power' for horses and oxen as well as fodder for livestock. Extra hay was therefore often sold by farmers as a cash crop (Simpson, Hunter and Jefferson 1996). In the years following the Second World War, food shortages, government and later EC policies combined with technological advancement have given rise to greatly increased production from grasslands. In the forty years to the mid 1980's, output of beef and lamb doubled and that of milk more than trebled, whilst the total area of grassland used fell (Hopkins and Hopkins 1994).

This agricultural improvement of meadows has involved the widespread use of inorganic fertilisers. Many old meadows have been ploughed and re-seeded with short-term leys dominated by *Lolium perenne*, and have been given large applications of inorganic fertiliser, especially nitrogen. Applications of nitrogen, phosphorus and potassium whilst increasing the productivity of meadows, in terms of the hay crop, have dramatic negative effects on the plant species diversity. This effect has been well documented in trials on mesotrophic and other grassland communities (Coleman *et al.,* 1987; Mountford *et al.,* 1994; Silvertown *et al.,* 1994; Smith, Shiel and Millward 1998). From the study of data from the Park Grass Experiment, Silvertown (1980) suggests that there is a generality about this relationship that is independent of the actual species involved.

Despite the impact of wartime ploughing and subsequent agricultural improvement, nearly 60% of old grassland that existed in 1939 remained unploughed 20 years later (Bakker 1960; in Hopkins and Hopkins 1994). During the twenty years that followed, management changes had a major impact on the remaining core of old grassland. The average use of nitrogen fertiliser on grassland trebled. The practice of making silage, together with the fertiliser and reseeding practices which accompany it has probably had more effect on species diversity than any other factor (Hopkins and Hopkins 1994).

The widespread adoption of silage production by farmers has also led to a reduction in the traditional farming practices that help to maintain the botanical composition of hay meadows. The production of silage which involves the cutting and collection of grass without the need for drying in the field was attractive to farmers for a number of reasons. It is not dependent on good weather and the early (or multiple) cutting of herbage means conservation of plant material can occur when it is in a more digestible stage of growth. It is also less reliant on man power and came in line with increasing farm mechanisation. Finally, silage can be made from more succulent heavily fertilised swards.

Management practices to encourage the restoration and survival of remaining old meadows have become increasingly important in recent years. Agricultural policy in Europe has placed a greater emphasis on incorporating environmental objectives (Hopkins *et al.,* 1998). For example, the introduction of Environmentally Sensitive Areas (ESA) in 1987 has provided opportunities for farmers to manage their land according to locally defined parameters designed to benefit wildlife and landscapes (Coates 1997).

1.2 Northern Hay Meadows

The traditional northern hay meadow, once a colourful and widespread feature of the Pennines is now mainly restricted to a few dales in the north of the region; North Yorkshire, Durham and East Cumbria at an altitude of between 200 and 400 m. Examples are also found in the Lake District and towards the south in Lancashire as well as to the north in Northumberland (see Figure 1). This distinctive plant community is classified as *Anthoxanthum odoratum – Geranium sylvaticum* grassland (MG3) within the framework of the National Vegetation Classification (Rodwell 1992).

The NVC draws a distinction between different types of northern hay meadow. The *Briza* sub-community has a richer flora and is considered 'better' than the *Bromus* sub-community, in nature conservation value. This is because they are considered to be the less agriculturally improved type. The *Bromus* sub-community is often associated with those fields that were ploughed during the Second World War, though not necessarily fertilised with chemicals (Rodwell 1992).

1.2.1 Extent

The total area of this vegetation type remaining is thought to be in the region of only 600 ha recorded from as many as 300 sites (Rodwell and Cooper 1995). The scattered nature of the vegetation type is highlighted by the fact that Cumbrian surveys have shown ~10% of the total area occurs as roadside verge with an average stand size of just 0.2 ha (Clayden and Slater 1986; Densley 1987).

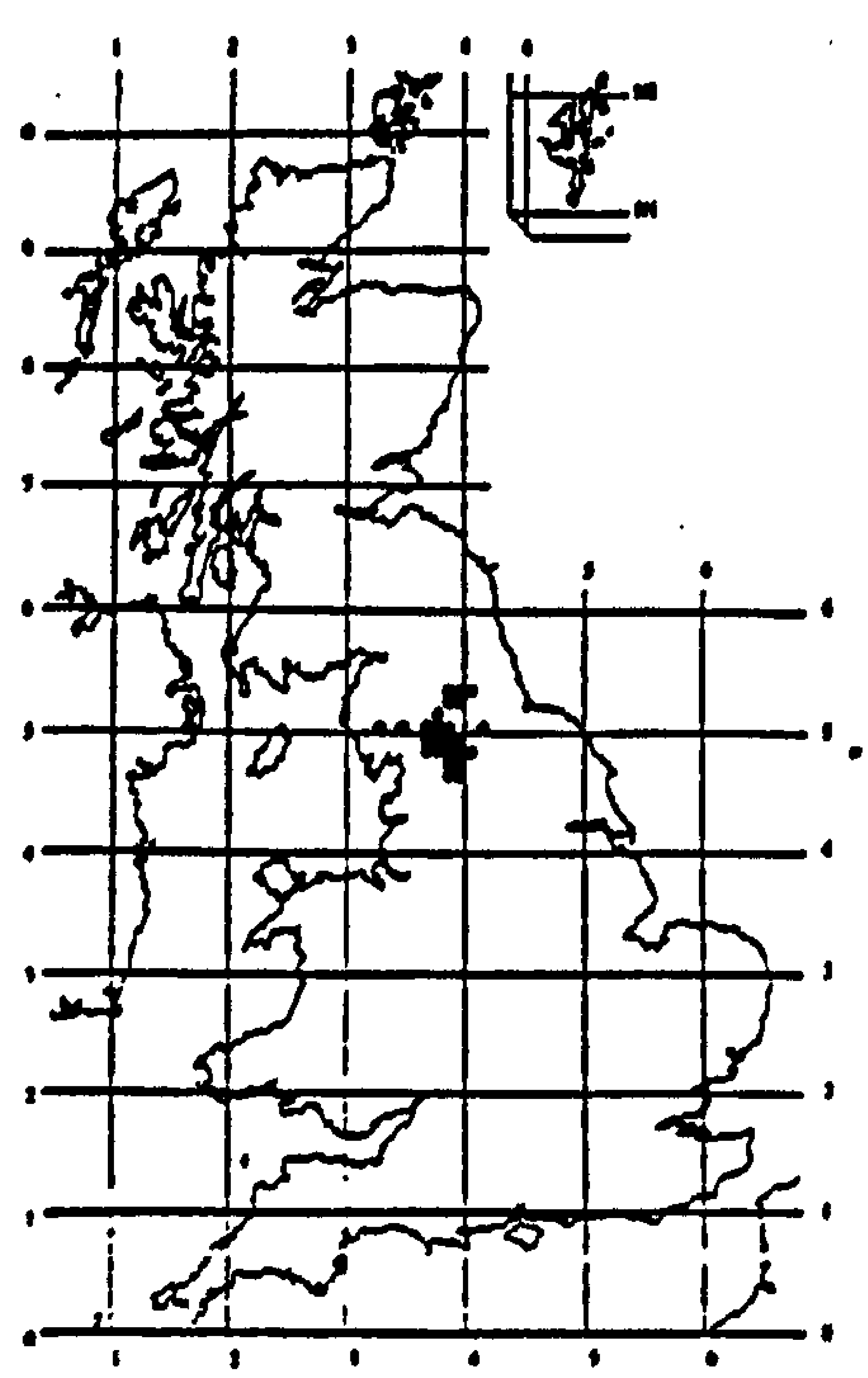


Figure 1. The distribution of MG3 communities. Reproduced from Rodwell (1992

1.2.2 Productivity

In general, meadows are to be found on the best land of a farm because of the need to provide feed for over-wintering livestock. On farms with a high proportion of good land the distinction between meadows and pastures tends not to be fixed however, where the proportion of good land is small then the same areas of land may have been used as meadows for several generations (Hopkins 1990). This latter situation is generally the case in the Pennines, where due to the topography of the farms there is frequently only a small proportion of the total area suitable for hay production.

Despite the short growing season and lack of agricultural improvement the herbage production of the MG3 type meadows is considerable. Jones (1984) measured a herbage production of 3.2-3.8 tonnes dry matter (DM) ha^{-1} in Upper Teesdale whilst Edwards (1999) measured yields of between 4.3 and 8.2 tonnes DM ha^{-1} in meadows at Bowber Head and Piper Hole in east Cumbria. These herbage production levels were achieved in a maximum growing period of 10 weeks at most between 20th April and the 15th July. The length of time allowed for the growth of herbage (from shut up to hay cut) was one of the main factors controlling the size of the hay crop.

Despite the visual dominance and apparent high proportion of dicotyledonous herbs such as *Geranium sylvaticum, Sanguisorba officinalis* and *Cirsium helenoides* within the sward, it has been shown that grasses form the major part of the herbage mass (Edwards 1999). This high proportion of grasses is seen as crucial to the quality of the forage.

1.2.3 Timing of management operations and climate

The timing of the hay production operations within Pennine meadows, and the distinctive floristics of the meadows, is closely linked to the climate of the area (Piggot 1956). Manley (1936) summarised the climate as cold, wet, windy and cloudy. These conditions make the growing season within the area shorter than any other agro-climatic zone within England and Wales (Smith 1976). The winters are long and long-lying snowfalls are common (Manley 1940). Recent years have seen a succession of relatively mild winters which, if part of a more long-term pattern, could have worrying consequences for the ecology of upland grasslands (Akinola *et al.*, 1998).

Rodwell and Dring (2001) used data contained within the diaries of Walter Umpleby during his 43 years (1954-1996) at New House Farm, Malham, and data from the weather station at the Malham Tarn Field Studies Council Centre to study how weather affected the timing of management operations on this traditionally managed farm. It was possible to see how the timing of hay making operations and the weather were linked. The main findings of interest were:

1. Whilst yearly variation is considerable, it is generally not until mid-late April that the daily mean temperature exceeds that necessary to maintain plant growth. Frost often continues until late March and sometimes into April. By mid October temperatures have generally dropped below this threshold again.
2. Even during the mid summer period when the hay is being cut, the mean daily sunshine duration is often below 5 hours and rarely exceeds 6.5.
3. A wet, late winter and spring, and sustained wet weather during the growing period causes a postponement of hay making whilst warm sunny weather speeds the completion of hay making.

During the winter months and in early spring, the meadows are grazed by sheep when there is sufficient grass, it is also during this time that farmyard manure will be applied when the ground is sufficiently dry. Cattle are generally not grazed in the meadows during the spring as they would cause too much damage to the sward through poaching. The grazing is then halted from late April and the meadows "shut-up" to allow the uninterrupted growth of herbage for subsequent conservation as hay.

Studies which have looked at the long term trends in the timing of hay cut dates in meadows on Pennine farms (Smith and Jones 1991; Rodwell and Dring 2001) have shown that there is great variation in the date of commencement of the hay cutting operations. In particular from 1954-1996 at New House Farm, Malham, hay cutting commenced on dates stretching from 12th June to 20th July, and the ending of the hay cut was between 3rd July and 19th October. This was mainly due to the need to wait for dry weather to carry out the process. There is some evidence that with the increased mechanisation of hay-making the start dates have remained the same but the hay-making as a whole has been compressed into a shorter period of time (Smith and Jones 1991).

Following the hay cut, the meadows are generally quickly grazed by sheep so that the herbage in the uncut edges of the meadows is utilised. The subsequent growth, throughout the late summer and autumn, frequently called the aftermath, will then be heavily grazed by either cattle or sheep.

1.2.4 Biodiversity Value

The 1992 European Union Habitats Directive Annex 1 recognises this community as mountain hay meadows (British types with *Geranium sylvaticum*) and states that; "The floristic composition of mountain hay meadow vegetation in the UK is unlike that found in the rest of Europe and appears to be unique in the EC."(Brown *et al.*, 1996). Many surviving stands of the vegetation have been designated Sites of Special Scientific Interest (SSSI), the UK Biodiversity Action Plan (Anon 1994) has also added impetus to conservation of the remaining meadows. The Pennine Dales Environmentally Sensitive Area (ESA) scheme and the Countryside Stewardship Scheme in the region both have increasing the biodiversity of grasslands as priority.

The *Anthoxanthum odoratum- Geranium sylvaticum* meadows are the major remaining habitat for species such as *Geranium sylvaticum, Sanguisorba officinalis, Cirsium helenoides* as well as some of the rarer species of *Alchemilla* as described by Bradshaw (1962).

Meadows provide a rich feeding ground for many birds. In spring waders such as lapwing, curlew, redshank and snipe use meadows for feeding and breeding (Ratcliff 1990). Bains (1988) demonstrated a marked decline in species of waders in areas where improvement had taken place whilst Standen (1984) showed that changes taking place within the invertebrate communities of meadows were associated with improvement.

1.2.5 Agri-Environment Support

There are two main schemes which provide farmers with financial support for northern upland hay meadow management. These are the Countryside Stewardship Scheme and the Environmentally Sensitive Area Scheme (ESA). A majority of meadows in North Yorkshire, Durham, Northumberland and Cumbria fall within the Pennine Dales and Lake District ESA. Both of these ESA have tiers with management prescriptions specifically aimed at the enhancement of hay meadows. The Countryside Stewardship Scheme also provides payments for positive traditional management.

1.3 Factors affecting botanical diversity in meadows

A considerable amount of work has been carried out looking at how certain aspects of management affect the composition of meadow swards. Such work began with the Park Grass Experiment in 1865 (Jenkinson *et al.,* 1994; Silvertown *et al.,* 1994). Due to conservation interest of these habitats this work has been continued in wildflower rich meadows on the Somerset Levels (Kirkham and Wilkins 1994) as well as in the Pennine Dales (Jones 1984; Smith and Jones 1991; Smith, Shiel and Pullan 1996; Smith *et al.,* 1996 and Smith *et al.,* 1998).

1.3.1 Inorganic Fertiliser

There have been numerous studies looking at the effects of inorganic fertilisers on the composition of meadow swards, including the Park Grass Experiment (Silvertown 1980), the Somerset Levels (Kirkham and Wilkins 1994; Mountford *et al.,* 1994) and the Pennine Dales (Smith *et al.,* 1996; Smith *et al.,* 1998).

Analysis of the Park Grass Experiment by Silvertown *et al.*, (1994) showed a strong relationship between sward productivity and reduced floristic diversity. In particular an increase in the dominance of grasses over herbs was shown to be associated with higher productivity.

Kirkham and Wilkins (1994) reported an increase in yield from 4.7 t ha^{-1} to 10.5 t ha^{-1} in a herb rich meadow in Somerset when 200 kg nitrogen, 75 kg phosphorous and 200 kg of potassium ha^{-1} were added. Jones (1984) showed an increase in yield of around 35-50% when 376.3 kg ha^{-1} of NPK fertiliser (75:37:37) was applied to meadow plots in the Upper Teesdale area of northern England. However these increases in productivity are also associated with rapid declines in the botanical diversity.

The interdependence of species richness and productivity is one of the more general biodiversity patterns (Grime 1979; Tilman 1993). General theories on the relationship between standing crop biomass and species diversity predict a unimodal relationship, with an optimum at intermediate levels of diversity (Grime 1979). The level of diversity that is the most productive, is however dependent on the vegetation type and numerous other factors relating to the locality of that vegetation (Olff and Bakker 1991).

It is accepted that soil nitrogen supply primarily limits herbage yield. There is a linear relationship between increased nitrogen fertiliser application and yield up to about 250 kg N ha^{-1} with further diminishing marginal returns up to around 530 kg N ha^{-1} possible. Yield increase reaches a maximum at around 5.2-14.3 t ha^{-1} and then progressively declines at very high fertiliser application rates (Morrison 1987 cited in Smith and Rushton 1994).

It has been suggested that, as productivity rises competition in the shoots increases as light rather than nutrient availability becomes a limiting factor (Vermeer and Berendse 1983). As light is a unidirectional resource, fast-growing perennials with a tall stature dominate high nutrient habitats (Aerts 1999). In the case of mesotrophic grasslands perennial grasses such as *Lolium perenne* or *Arrhenatherum elatius* would become dominant.

Whilst the amount of nitrogen available in the soil is the main factor influencing both plant growth and botanical diversity, it has also been shown that the highest number of species occurs in grasslands when soil phosphorus is below the optimum for plant growth, 5-8 mg P 100 g^{-1}, (Janssens *et al.,* 1998). Because phosphorus is less labile (less prone to leaching) than nitrogen, high phosphorus levels are believed to inhibit any desired increase in species diversity when traditional meadow management is re-established on a site which has previously been intensively managed. In contrast potassium is at an optimum for plant growth (20 mg 100 g^{-1}) when species diversity is highest.

1.3.2 Grazing Management

Farmers attempt to maximise farm profitability by managing grasslands for optimum yield (Wilkins and Harvey 1994). In meadows this involves grazing by livestock both after hay cut and throughout the winter and early spring until the meadows are shut-up once more. This grazing is an important factor in reducing the dominance of competitive species and in creating germination opportunities through the trampling effect of the stock (Grubb 1977). The timing of these operations is very much dependent on regional variations in climate and annual differences in weather conditions.

The cessation of grazing has been shown to affect species composition in a range of differing grassland types (Doleman and Sutherland 1992; Gibson and Brown 1992; Smith and Rushton 1994). Smith and Rushton (1994) showed that changes occurred in traditionally managed meadow vegetation when grazing management was altered. In their experiments grazing was prevented for the entire year, or for autumn or for the spring. All of the treatments changed the composition of the vegetation after four years. The treatment which excluded grazing altogether caused a marked reduction in species richness, whilst the changes shown in the other treatments were mainly due to changes in relative abundance of species. Preventing grazing in the spring favoured certain grasses (in this case *Anthoxanthum odoratum, Lolium perenne, Poa trivialis* and *Cynosurus cristatus*) whilst preventing grazing in the autumn favoured the herbs (*Geranium slyvaticum, Cirsium helenioides* and

Sanguisorba officinalis). Subsequent work in meadows has shown that these responses are increased when combined with other factors such as fertiliser use and hay cutting date (Kirkham and Wilkins 1994, Smith Shiel and Pullan 1996; Smith *et al.*, 1996).

1.3.4 Hay Cut Date

The date at which the hay crop is cut is an important factor affecting species diversity. Management guidelines for Environmentally Sensitive Areas (ESA) restrict the early cutting of meadows in order to enable ground-nesting birds to rear their young. However it is also thought that this practice will enable later flowering herb species to set seed.

In a Pennine hay meadow it has been shown that either early (14th June) or late (1st September) cutting encourages competitive grass species, increasing their yield and reducing plant species diversity (Smith *et al.,* 1998). Thus wildlife conservation of mesotrophic meadowland requires the joint implementation of appropriate cut date and grazing regimes, as well as limiting inorganic fertiliser applications.

1.4 Farmyard Manure

The production of farmyard manure is an inevitable consequence of the traditional animal production system found in the Pennine Dales. The livestock, generally cattle, are housed in barns over the winter period although sheep may sometimes be housed as well. The animals are generally housed on straw bedding material bought in from farms with arable production. This material becomes soiled with faeces and urine and is then removed from the barns and stored outside in middens.

The continual 'mucking out' of animals produces a stock pile of manure, the size of which will largely depend on the number of animals kept. In the traditional system this will be related to the productivity of the meadowland on the farm as the ability to feed over wintering stock is the main limit to stock numbers. The feeding of more concentrate feed to animals in more intensive systems may affect this balance.

In order to maintain the productivity of the meadows, the manure is spread onto the meadowland, and pastures, in the winter or early spring when the ground is sufficiently dry. The manure will have been stored for varying periods of time before this occurs. Manure has been used to maintain soil nutrients and therefore hay production for centuries (Simpson, Hunter and Jefferson 1996).

It is well established that farmyard manure can play a role in increasing soil fertility and reducing botanical diversity when applied at high levels (Williams 1978). Its influence when used at modest rates on species-rich meadows is less clear.

The impacts of other elements of hay meadow management have received a considerable amount of attention. For example inorganic fertiliser application (Silvertown 1980; Kirkham and Wilkins 1994; Mountford *et al.*, 1993; Smith *et al.*, 1996; Smith *et al.*, 1998), hay cutting dates (Smith *et al.*, 1998; Rodwell and Dring 2001) and grazing management (Smith and Rushton 1994).

It is possible that, aside from the issue of nutrient supply, farmyard manure may contain viable seeds (Thill and Mallory-Smith 1997; Rupende *et al.*, 1998) which could influence meadow diversity when manure is spread. In order for a species to colonise vegetation, it requires a propagule to be dispersed and for that propagule to arrive at a site suitable for germination (Grubb 1977). Manure may help to provide such a medium for dispersal as well as a suitable niche. The role of this thesis is therefore to explore the possible mechanisms by which farmyard manure may influence botanical diversity in traditionally managed meadows in this manner.

2. Literature Review

Farmyard manure has traditionally been applied to most hay meadows to compensate for the removal of soil nutrients through hay cutting. In the traditional upland farming system of the Pennines hay cut from the meadows in the summer is fed to the stock that are housed over winter in the barns. The dung produced is removed with the bedding material, usually straw as farmyard manure. Farmyard manure is applied to the meadows between autumn and spring when the land is dry enough. Until 1939 animal manures provided the main source of nitrogen, phosphate and potash in the UK (Archer 1985).

Farmyard manure application to botanically diverse swards has been reported to decrease the dicotyledonous component of the sward whilst increasing the graminaceous component (Silvertown 1980). However, farmyard manure provides a slower release of nutrients generally at lower rates than inorganic fertilisers. Long term fertiliser experiments such as the Park Grass experiment at Rothamsted suggest that whilst large applications of farmyard manure can reduce botanical diversity, continued cropping of unfertilised grassland will also lead to reductions in diversity (Williams 1978). The traditional management carried out over generations at many farms show it is also possible to maintain a diverse sward through appropriate levels of farmyard manure application (Smith *et al.,* 1996).

Different authors have suggested differing levels of manure application to retain fertility on species rich grassland. Crofts and Jefferson (1994) suggested applying 20 tonnes ha^{-1} in one dressing every three to five years on grasslands of high nature conservation value, whilst the conditions for the Environmentally Sensitive Area scheme state that a maximum of 12.5 tonnes ha^{-1} should be applied in a single dressing per year. Providing a clear recommendation based on historical rates is difficult due to a lack of available data (Simpson, Hunter and Jefferson 1996). Also specific conditions at sites can alter the level needed.

Whilst it is an integral part of hay meadow management the use of farmyard manure will not have been consistent on meadows. Changes in the stocking rate of farms would have been matched by application rates of manure. Indeed, even within a farm, the meadows closer to the buildings may have been easier to fertilise, as the putting out manure by hand would have been an extremely time consuming and laborious task. The use of modern manure spreading equipment may well have reduced much of this variation in application rates.

Another factor that creates difficulty in drawing up precise guidelines for the use of farmyard manure is the variability of different types. For example a number of factors can affect nutrient content of farmyard manure. These include:

1. The species of animal producing it.
2. The age and condition of those animals.
3. The food consumed by the animals.
4. The type and amount of bedding used.
5. Storage of the manure.

Simpson, Hunter and Jefferson (1996).

Manure is never homogenous even from one animal on one type of bedding. It can be expected that manure in some cases may not be the same today as it once was given better animal feeding. This highlights the need for appropriate feeding controls over animals in farming systems that are aimed at maintaining the botanical diversity of meadowland. Also the use of concentrate feeds may have enabled the maintenance of higher stocking rates giving rise to increased quantities of manure available for application. The need when assessing management of hay meadows is therefore to look at the complete farming system rather than just meadows is isolation.

Once collected from barns the manure will be stored for varying lengths of time before it is spread. During this time certain changes in the manure will occur. When considering these it is important to distinguish between stock piling (undisturbed storage) and composting (usually involving aeration by disturbance). To allow good composting the midden needs to be regularly turned to produce a relatively dry material. The lack of mechanisation in most of the small farms in which these meadows occur means that generally the manure has been stored rather than composted. During manure storage the carbon:nitrogen ratio will decrease. The more straw bedding found in manure the higher the initial C:N ratio.

The vegetation found within traditionally managed hay meadows of the Pennines is noted for its botanical diversity (Ratcliff 1977; Rodwell and Cooper 1995). These complex, dynamic plant communities are thought to be relatively stable over the long term (Tansley 1939; Rodwell 1992). In order for this to be the case the species found within them need to be able to co-exist. A key factor in maintaining species rich vegetation is the ability of its constituent species to reproduce and thereby maintain their populations. Such reproduction can occur through the dispersal and subsequent germination of seed or via vegetative reproduction.

The potential role that farmyard manure plays in dispersing the seed of UK hay meadow plants is unknown (Simpson, Hunter and Jefferson 1996). Neither is it known how disturbance to the sward that manure application can cause effects the germination of seed. Hay from a meadow with a diverse sward will contain a large quantity of viable seed (Wells, Frost and Bell 1986). The extent to which this seed is returned to the meadow when manure is spread is unknown. This seed could either germinate immediately or become incorporated into the soil seed bank from which it can germinate at a later date. It is also not known whether certain species may be favoured by this process. A thorough understanding of the role that seeds play within ecosystems is critical for understanding how to conserve endangered communities or restore those communities (Chambers and MacMahon 1994). It is only through a complete knowledge of how seeds disperse and germinate within an ecosystem that it becomes possible to maximise establishment and persistence of desired species.

2.1 Seed Dispersal

The dispersal of plants is of fundamental importance in contributing to successional change or the maintenance of plant populations. In practice it is however very difficult to follow the fate of seeds after they have left their parent. Thus seed dispersal remains one of the least understood processes of plant life cycles (Schott

1995; Eriksson and Jakobsson 1999). If the dispersal of offspring increases the fitness of a parent, it is to be expected that dispersed offspring survive and reproduce better than undispersed offspring. The main factors that favour dispersal are avoidance of natural enemies or sibling interactions and the increased likelihood of being in a suitable establishment site (Wilson and Travest 2000).

The unit of dispersal may be a fruit, group of fruits or other units of seeds and associated dispersal structures, therefore when considering the dispersal the correct term should technically be diaspore or propagule (Wilson and Traveset 2000) however the more widely used term 'seed' will be used here. Dispersal dynamics of single species have generally shown that the majority of seeds produced by plants reach the ground very near to the parent plant (Jefferson and Usher 1989). The resulting patterns of seed deposition are usually leptokurtic, a distribution represented either by a negative exponential function or by a curve that peaks a short distance from the plant and then shows a negative exponential decrease (Wilson 1993). This spatial distribution of dispersed seeds around their source is known as the 'seed shadow' (Janzen 1971). The dispersal shadows described are well studied within individual populations (Wilson 1993) but are less well studied for entire plant communities (Rabanowitz and Rapp 1980; Chambers 1994). The majority of these studies have concentrated on the role of the wind in dispersing seeds. The dispersal of seeds over longer distances by either biotic or abiotic agents are less well studied. This is despite the fact that the tail of the dispersal curve may be at least as important as the modal part of the curve (Wilson and Travest 2000). Therefore these longer distance dispersal events could be of considerable importance in structuring some plant communities.

Important dispersal mechanisms in grasslands include wind, animals and ballistic seeds (Chambers and MacMahon 1994), however dispersal by agricultural practices may also be important. Within the context of Pennine hay meadows it would appear that the main methods by which seed can disperse into the vegetation over longer distances would be via wind dispersal from plants in surrounding vegetation, the dispersal of plants by mechanical spread during hay cutting and turning operations (Smith, Pullan and Shiel 1996), dispersal on farm machinery (Bakker *et al.*, 1996), dispersal on farm animals or exozoochorous dispersal (Fischer *et al.*, 1996) and dispersal within farmyard manure when it is spread (Dastghieb 1989). It is possible that manure when spread would contain both seed ingested by animals and seed that has not been ingested.

2.1.1 Seed dispersal by management practices

In certain ecosystems the actions of man have greatly influenced seed dispersal and the establishment of species. Agricultural practices in particular have been shown to influence the dispersal of seeds (Bakker *et al.*, 1996). For example arable weed species such as *Agrostemma githago* and *Melampyrum arvense* are now extinct on arable fields in Central Europe because of the use of cleaned seed in arable production (Schneider *et al.*, 1994 in Bakker *et al.*, 1996 and Poschlod *et al.*, 1996b; in Bakker *et al.*, 1996). It has been shown that harvesting machinery can also be involved in the dispersal of arable weeds (Howard *et al.*, 1993; Mortimer *et al.*, 1993). Renewed interest in organic fertiliser use has also highlighted the fact that farmyard manure application can cause weed introduction to arable land (Mt Pleasant and Schlather 1994; Rupende *et al.*, 1998).

In grassland systems dispersal of seed by hay making machinery is known to be an important dispersal vector when all fields are interconnected by the annual mowing scheme (Strykstra *et al.*, 1996; Strykstra and Verweij 1997). It would also be logical to conclude that manure applications to grassland could be involved in seed dispersal.

Despite this wide range of study Poschlod (1996b; in Bakker *et al.*, 1996) noted that there is a lack of information about how a distinct management process can disperse seed in grassland systems and Bakker *et al.*, (1996), whilst stating that seed can be dispersed by grazing animals and cutting machinery, note that this knowledge has not been incorporated into management practices.

2.1.2 Reproductive strategies in Pennine meadows

The high levels of botanical diversity within Pennine hay meadows means that no single mechanism of dispersal and reproduction will be suitable for all or even a majority of species. Grime, Hodgson and Hunt (1988) describe five main reproductive strategies in the British Isles. These are shown in Table 2.2.1.

Table 2.2.1 Reproductive strategies of plants. Reproduced from Grime Hodgson and Hunt (1988).

Strategy	**Functional characteristics**	**Conditions under which the strategy appears to enjoy a selective advantage**
1 Vegetative expansion.	New shoots vegetative in origin and remaining attached to the parent plant until well established.	Productive or unproductive habitats subject to low intensities of disturbance.
2. Seasonal regeneration.	Independent offspring (seeds or vegetative propagules) produced in a single cohort.	Habitats subject to seasonally predictable disturbances by climate or biotic factors.
3. Persistent seed or spore bank.	Viable but dormant seeds or spores present throughout the year; some persisting more than 12 months.	Habitats subjected to temporally unpredictable disturbances.
4. Numerous widely dispersed seeds or spores.	Offspring numerous and exceedingly buoyant in air; widely dispersed and often of limited persistence.	Habitats subjected to spatially unpredictable disturbance or relatively inaccessible (cliffs, walls, tree trunks etc).
5. Persistent juveniles.	Offspring derived from an independent propagule but seedling or sporeling capable of long-term persistence in a juvenile state.	Unproductive habitats subjected to low intensities of disturbance.

Of the categories given in Table 2.2.1, the first three are common within meadows whilst four and five are unlikely to be encountered. Vegetative expansion through the formation of features such as stolons and rhizomes is likely to be an important strategy for a number of prominent perennial species which are found within Pennine meadows. For example *Filipendula ulmaria* is noted for its ability to spread by rhizomatous means (Grime, Hodgson and Hunt 1988) and *Geranium slyvaticum* is also capable to reproduction by rhizome spread (Rodwell 1992). However, other perennial herbs are known to mainly reproduce by seed, such as *Centaurea nigra* (Grime, Hodgson and Hunt 1988). There are also a number of perennial grass species within hay meadows that can reproduce by vegetative means such as *Poa trivialis* and *Lolium perenne*. These species however are also capable of production of large quantities of seed which can readily germinate into gaps or be incorporated into the soil seed bank (Grime, Hodgson and Hunt 1988).

The majority of the species within meadows are capable of vegetative spread and germination from seed either immediately following seed shed or later from a transient or long term soil seed bank. However, a few species of annul plant are likely to be encountered such as the herbs *Myosotis discolor* or *Rhinanathus minor* and the grass *Bromus hordeaceus* which rely on germination from seed. The lack of a persistent seed bank for *Rhinanthus minor* means that it relies on seed being produced each year and being able to germinate the following spring. It is for this reason the species is considered a good indicator of long term traditional management.

2.2 Factors affecting species colonisation.

Following the dispersal of seed it is necessary to understand the factors which may affect its recruitment to the population. The germination and subsequent establishment of plants can be affected by a number of factors.

2.2.1 Disturbance

Disturbance is often the process which leads to the creation of a gap which can be defined as a 'plant free space' or a competition free space' (Bullock 2000). The gap concept is central to plant ecology especially in attempting to explain species co-existence and community structure (Tilman 1993; Lavourel and Chesson 1995; Pacala and Levin 1997). The basis of these explanations is the idea that species within a community respond differently to gaps, in terms of their ability to colonise and in terms of their requirement for gaps for regeneration (Bullock 2000). The nature of the gap will also determine the relative importance of different sources of seed in colonising the gap (Thompson 2000).

A considerable amount of research has focussed on the conditions that help to facilitate the invasion of plant communities by non-native species. Some of this research has looked at the role of disturbance and nutrient supply. Given that the basic processes that admit exotic plants are essentially the same as those which allow regeneration within a plant community (Davis *et al.,* 1998) it is useful to examine this work.

A colonising species will have more success if certain conditions are met. Firstly there must be an availability of propagules. There should also be a reduction in competition and or a reduction in the use of resources (Davis *et al.,* 2000). An invading species will have more success if it does not encounter intense competition from resident species. This assumption is based on the theory that competition intensity should be inversely correlated with the amount of unused resources (Davis *et al.,* 1998).

It has been proposed that disturbance facilitates invasions by eliminating or reducing the cover, or vigour of competing plants or by increasing resource levels (Hobbs 1989; D'Antonio 1993). Disturbance creates a reduction in the need for resources within a plant community. These resources could be light, nutrients in moisture for example. Higher soil temperatures, light fluxes and soil moisture on disturbed soils or in open areas often favour seed germination (Baskin and Baskin 1989).

The theory of fluctuating resource availability proposed by Davis *et al.*, (2000) predicts:

1. Environments subject to pronounced fluctuations in resource availability will be more prone to invasions.
2. Environments are more susceptible during periods immediately following an abrupt increase in supply or decrease in demand of resources.
3. Invasibility will increase following disturbances that increase resource supply or decrease resource demand.
4. Invasibility will increase when there is a long interval between the increase in supply of resources and recapture of them by resident vegetation.
5. Grazers increase invasions
6. There is not necessarily a relationship between species diversity and invasions.
7. There is no relationship between community productivity and invasions.

2.2.2 Sources of disturbance

Whilst sources of disturbance within hay meadow vegetation may include the action of animals such as moles or natural events such as drought or frost heave the action of grazing animals either directly through poaching of the sward or defecation are likely to be most important. Defoliation, trampling and excretal return can all cause plant damage or death (Marriot *et al.,* 1997). The size and range of these patches may vary from as little as 1-2 cm in diameter (Silvertown and Smith 1988) up to 10-20cm when created by dung patches (Castle and MacDaid 1972). Grazing by cattle and sheep within Pennine hay meadows is likely therefore to produce niches for the germination and or vegetative spread of the plant species present; it is also likely however that the application of manure to meadows may provide suitable niches for the regeneration of meadow plants.

Manure application is one form of disturbance that could create a gap in the sward. The application of manure to meadowland using muck spreaders often creates an uneven covering of manure, larger clumps of relatively wetter material are delivered to some patches in the sward. Despite a lack of direct evidence it could be expected that these patches of manure can lead to a depression of growth early in the growing season. In this manner gaps in the vegetation would be likely to be formed. Indeed Aikmen (1884) noted the difficulty of spreading cow manure evenly as well as the manner in which it resists decomposition for long periods. These gaps within the sward could be filled in a number of ways. Surrounding plants could spread by vegetative means into the gap, viable seed from within the soil seed bank could germinate and establish within the gap or seed could germinate from within the manure itself and establish in the gap.

In a study from Northern Ireland the association between cattle slurry and *Rumex obtusifolius* populations in grassland was shown to be most probably due more to favoured establishment of seedlings following slurry application, which created gaps in the vegetation, rather than dispersal of seed within slurry (Humphreys *et al.*, 1997).

The micro-environmental conditions within a gap produced by manure would be likely to be different from a similar sized gap produced by poaching from grazing animals for example (Malo and Suarez 1995a). Most species require specific environmental conditions to become non-dormant and then to germinate, therefore the conditions within the micro-sites into which the seeds are dispersed is likely to be an important determinant of seed germination (Chambers *et al.*, 1990). It could therefore be possible that some species found within hay meadows may be favoured by conditions found within those gaps produced by manure application.

As has been discussed in section 2.3.1 the disturbance combined with increased nutrient supply which manure application may supply could well aid the colonisation of meadow plants. These plants could germinate either from the manure itself or the soil seed bank. Indeed Burke and Grime (1996) carrying out work on invasions into a limestone grassland, found that invasion was highest at sites that were nutrient enriched and particularly when accompanied by disturbance.

Further evidence for this is given by Tallowin *et al.*, (1994). In an experiment looking at the successful colonisation of sown hay meadow plants disturbance increased the likelihood of establishment. It is possible therefore that the gap creating properties of manure application could have an effect in preventing the competitive exclusion of certain species within the sward. Factors which need to be taken into account when considering the effects of a gap in the sward produced by farmyard manure may included therefore; size of the gap, timing of creation of the gap and its longevity, the micro-climate found within the gap and the nutrients supplied by the manure. Due to a lack of research on the effects of manure in producing bare patches of ground much of this discussion will centre on research carried out on dung patches. Whilst not being the same the two are similar and certain general principles may apply to both.

It is well known that depositions of dung by cattle may kill vegetation. However the effects of manure application on production of bare ground patches has not been investigated. MacDiad and Watkin (1971) showed that 75% of plant material decayed rapidly beneath dung patches which had a mean size of $0.058m^2$ (Castle and MacDaid 1972). The consistency of manure is however different from that of dung and other factors such as storage time for example will affect this. It would however not be beyond possibilities to suggest that clumps of manure could produce gaps in the sward in the region of $0.01m^2$.

The persistence of gaps produced by farmyard manure application could be considered to be similar to those produced by dung deposition. In a temperate climate Castle and MacDaid (1972) found that dung persisted for up 55-63 days before crumbling. Cattle dung creates a crust which degrades slowly and only allows plant growth through it after 6 months (Williams and Haynes 1995). Whilst manure may degrade more quickly, MacDaid and Watkin (1971) showed that it took only 15 days for the vegetation under a dung patch to be killed. Any manure clumps would therefore only need to persist for a short period of time in order to create a vegetation gap.

Work on the colonisation of dung pats by Welch (1985) showed that most cow pats up to 2 months old were completely free from vegetation but none older than 30 weeks were bare. Spring deposited dung was colonised sooner than autumn dung. After 23 months autumn dung had significantly less plant cover than spring dung and after 3 years significantly more of the surface remained bare (Welch 1985).

Cattle dung created gaps have been shown to have lower moisture content and higher concentrations of phosphorus and nitrogen and favour germination of seed within the dung (Malo and Suarez 1995b). It could be expected that farmyard manure applications could act in a similar manner. Traveset *et al.*, (2001) noted that dung patches provide a higher level of water retention which may aid seed germination and or seedling survival. It has however also been shown that animal dung contains phenolic compounds and fatty acids which act as germination inhibitors for some plants (Marambe *et al.*, 1992).

The presumption that material accompanying viable seeds which have been consumed plays an important role in increased germination rate and or seedling survival has surprisingly little evidence (Traveset *et al.*, 2001). Whilst the chemical composition of cattle manure is known to be influenced by the diet of the animal producing it, the way the manure is collected, stored and handled and the bedding material used (Lekasi *et al.*, 2003) it is possible to obtain estimates of the nutrient content of cattle manure.

MAFF (1994) give the average values for fresh farmyard manure as shown in Table 2.2. Values for Nitrogen and Potash will reduce with storage.

Table 2.2. Typical nutrient content of fresh cattle farmyard manure. ***Available nitrogen shown in Table 2.3. (MAFF 1994)

	Total Nutrients			Available Nutrients		
Kg T^{-1} FYM	Nitrogen	Phosphate	Potash	Nitrogen	Phosphate	Potash
Cattle FYM	6.0	3.5	8.0	***	2.1	4.8

The nitrogen available to the sward depends on a number of factors and MAFF (1994) average values are given in Table 2.3.

Table 2.3 Percentage of total nitrogen available to next crop following application of fresh cattle manure. (MAFF 1994)

Timing	Autumn (Aug-Oct)		Winter (Nov-Jan)		Spring (Feb-April)
Soil Type	Sandy shallow	Other mineral	Sandy/shallow	Other mineral	All soils
Cattle FYM	5	5	10	15	20

2.3 Colonisation of gaps created by manure.

Once a gap in the vegetation has been created by farmyard manure application, providing an opportunity for the germination and establishment of meadow plants it will probably be filled by colonisation from a number of sources. The most obvious sources of colonising plants are from seeds found within the manure itself, those in the soil seed bank, seed rain and vegetative growth of plants surrounding the gap. These sources of colonisers are discussed in the next part of this chapter.

2.3.1 Factors affecting the seed content of farmyard manure.

Viable seed from hay meadows can become incorporated into farmyard manure when hay from the meadows is fed to stock housed in barns over the winter. It is possible that seed could enter the manure following digestion by animals or by falling directly into the manure from feed racks. Once in the manure the seed will need to remain viable during storage until it is spread onto the meadow. The next section of this chapter will look at the factors that could be involved in controlling how much seed of which species will be likely to be found within farmyard manure applications.

The species composition of the meadows from which the hay fed to stock is produced will be a major factor affecting the species composition of viable seed within farmyard manure. The northern hay meadow community, MG3 (Rodwell 1992), was shown to contain a mean of 26 species 2 m^{-2} with 35 species 2 m^{-2} in the more diverse *Briza media* sub community, whilst mean species richness values of 22 or 24 species m^{-2} were recorded by Alcock (1982) and Smith (1985) respectively. The constant species listed within the NVC (Rodwell 1992) are shown in Table 2.4.

Table 2.4 Constant species found within MG3 hay meadow vegetation.

	Constant Species
MG3 all sub-communities	*Plantago lanceolata*
	Rumex acetosa
	Ranunculus acris
	Geranium sylvaticum
	Anthoxanthum odoratum
	Conopodium majus
	Cerastium fontanum
	Dactylis glomerata
	Alchemilla glabra
	Trifolium repens
	Poa trivialis
	Festuca rubra
	Agrostis capillaris
	Holcus lanatus
	Sanguisorba officinalis
And in the Briza sub-community	
	Rhinanthus minor
	Bellis perennis
	Cynosurus cristatus
	Ranunculus bulbosus
	Leontondon hispidus

	Luzula campestris
	Trifolium pratense

When all the intermediate and occasional species are included 83 species of higher plant are listed as being present in the surveys conducted for the NVC in Pennine hay meadows.

It should be noted however that whilst sub communities of MG3 are the predominant vegetation types in these meadows, lack of uniformity gives rise to more than one plant community being found within individual meadows. Smith (1988) reports a survey in Teesdale which found 3 or 4 vegetation types within individual meadows. There are therefore a wide range of differing species which will be available for incorporation into hay made from traditionally managed sources in the Pennines. Figure 2.2.1 below (Rodwell 1992) shows the range of communities associated with MG1 *Arrhenatherum elatius* grassland. Although this diagram is based upon a type of lowland pasture it gives an idea of the range of different community types that could be found in and around Pennine hay meadows in addition to the MG3 sub-communities.

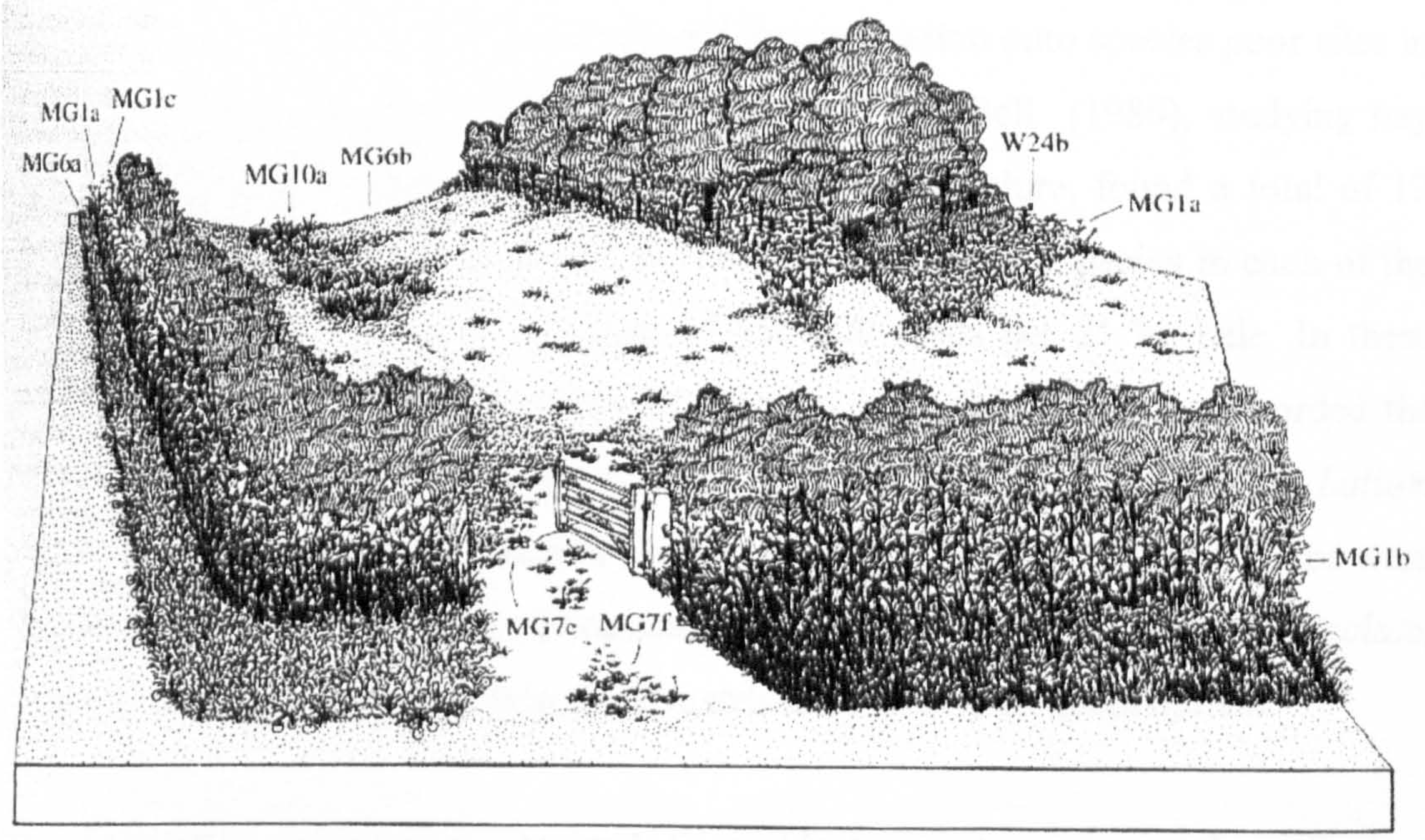


Figure 2.2. Typical pattern of grasslands in and around a run-down lowland pasture (Reproduced from Rodwell 1992).

MG1a	*Arrenatheretum, Festuca* sub-community on verge bank.
MG1b	*Arrenatheretum, Urtica* sub-community on disturbed verge.
MG1c	*Arrenatheretum, Filipendula* sub-community in verge ditch.
MG6a	*Lolio-Cynosuretum*, Typical sub-community on frequently mown verge edge.
MG6b	*Lolio-Cynosuretum, Anthoxanthum* sub-community with avoidance mosaic.
MG7e	*Lolio-Plantaginetum* towards gateway.
MG7f	*Poa-Lolietum* in gateway.
MG10a	*Holco-Juncetum*, Typical sub-community in ill drained field hollow.
W24b	*Rubus-Holcus* underscrub, *Arrenatherum-Heracleum* sub community invading around field margin.

The first step in the process of understanding if and how seed in traditional hay meadow systems can be dispersed by farmyard manure is to determine if viable seed is found in the hay which is initially cut from the meadows. A considerable amount of research has been carried out on this, as hay from species rich meadows has been seen as a potential source of seed for application onto species poor sites in order to enhance botanical diversity. Wells, Frost and Bell (1986), studying hay from a species rich lowland meadow in Cricklade, Wiltshire, found a total of 17 grasses and 24 herbs in 8 bales of hay. The mean number of species in each of the bales was 26 and in total an average of 450 000 seeds per 21 kg bale. In these samples, grasses accounted for 90% of the total seed. Of the grasses recorded the most abundant were *Dactylis glomerata, Festuca rubra, Holcus lanatus, Lolium perenne, Poa trivialis* and *Trisetum flavescens* whilst the most abundant herbs were *Ranunculus spp., Cerastium fontanum, Rhinanthus minor, Plantago lanceolata, Leucanthemum vulgare, Anthriscus sylvestris* and *Heracleum sphondylium.*

The spreading of material obtained from this hay onto a prepared seedbed of cultivated land gave rise to grassland containing 28 species, 18 of which were reported as originating from the hay (Wells, Frost and Bell 1986). Various other authors have reported success in using hay strewing as a means of increasing botanical diversity through propagule introduction in species poor swards (Atkinson *et al.*, 1995; Jones *et al.*, 1995; Mortimer *et al.*, 2002). A negative example of the same mechanism was given by Simpson, Hunter and Jefferson (1996) who suggested that Arkle Beck meadow in North Yorkshire a SSSI had a problem with the introduction of *Stellaria media, Bromus hordeaceus* and *Rumex* spp. from fodder bought in and fed to the stock within the meadow. Traditionally, the feeding of hay to stock within the field as well as the stacking of hay within the fields is also thought to be responsible for some of the wide-ranging differences in the local diversity of meadows (Bradshaw 1962).

Mortimer *et al.*, (2002) reported on an experiment using hay strewing to increase species richness of grassland in the Chilterns, southern England. Hay was taken from an *Arrenatheretum, Centaurea nigra* sub-community/*Festuca ovina-Avenula pratensis, Holcus lanatus-Trifolium repens* sub-community grassland (MG1e/CG2c) dominated by *Leontondon hispidus* with abundant *Carex flacca, Centaurea nigra, Festuca rubra, Lotus corniculatus* and *Sanguisorba minor* and spread at 20 t ha^{-1} onto a nearby species poor site. Significant increases in species richness (by 6 or 7 species) were subsequently recorded. Many of the species shown as colonising from the hay were perennial herbs especially *Centaurea nigra* whilst species failing to colonise were mostly graminoids, orchids and early flowering or low growing species. The date at which the hay is cut is crucial in determining the species of seed which may be found within hay (Wells, Frost and Bell 1986). Whilst Jones *et al.*, (1995) reported a cut date of 3rd of August from a donor meadow in Shropshire as aiding the dispersal and establishment of *Centurea nigra* in a recipient site, information on donor site cut date is absent from the Mortimer *et al.*, (2002) work.

However, Smith and Jones (1991) in a study of species phenologies in traditionally managed meadow in the Pennines at Bowberhead Farm, in Ravenstonedale, Cumbria found that out of the 16 species studied ripe seed is not present at the same time for all the species. Most species were shown to have little ripe seed before 1st July with *Sanguisorba officinalis, Centaurea nigra* and *Filipendula ulmaria* still without ripe seed by 21st August. These species would therefore be very unlikely to become incorporated as seed into hay from Pennine meadows except in years of exceptionally late hay cuts (see Figure 2.3).

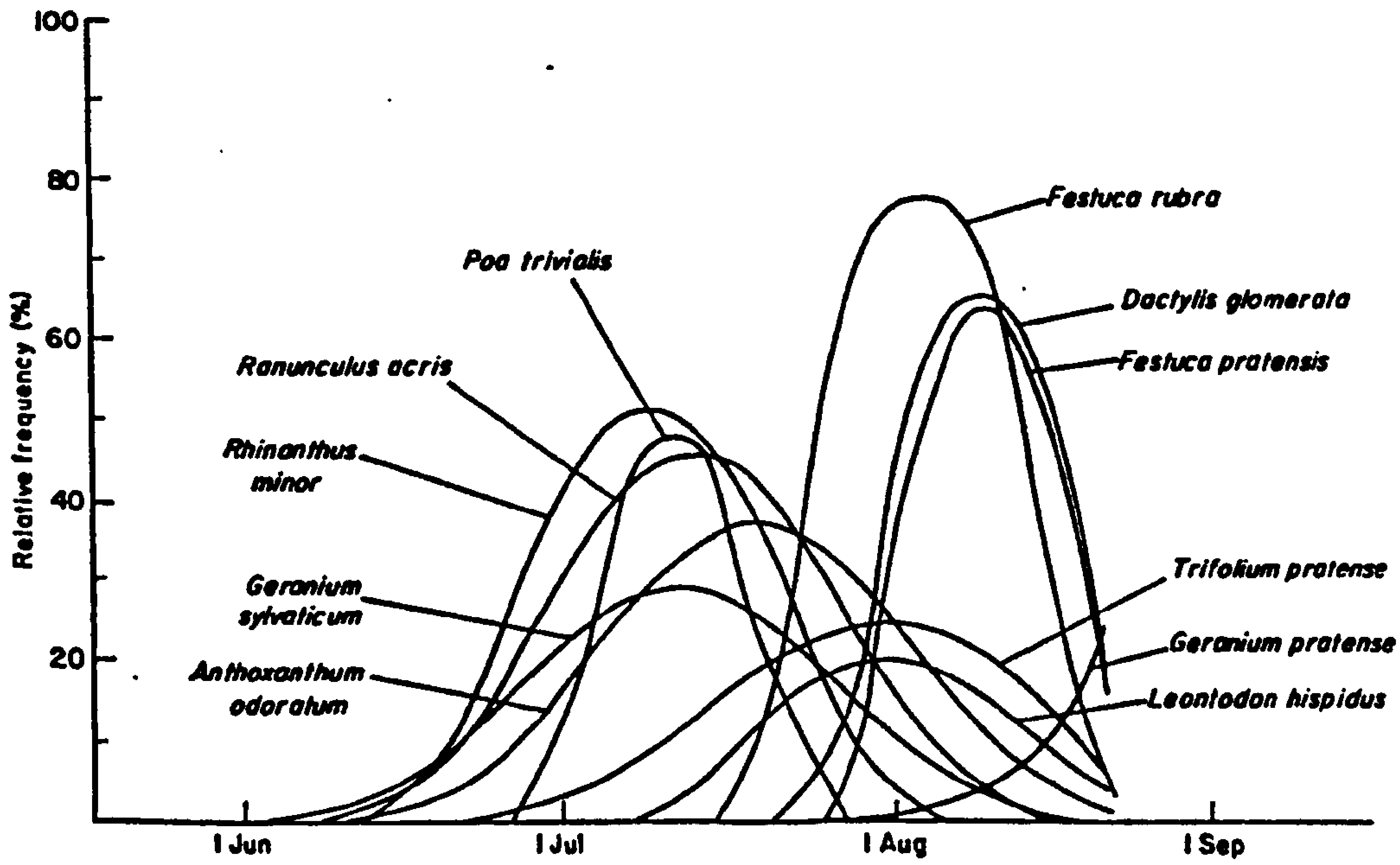


Figure 2.3 The development of ripe seed in 11 species found in a meadow at Bowberhead Farm, Ravenstonedale, Cumbria in 1988 (reproduced from Smith and Jones 1991).

Clearly ripe seed will be found in large quantities within hay from Pennine meadows it would however only contain those species which are mature at the hay cut date. Given the dates stated in Table 2.6 with hay cuts in the 1970's on average completed by 21st July the species such as those with the first peak of seed production shown in Figure 2.2, *Poa trivialis, Ranunculus acris, Rhinanathus minor, Geranium slyvaticum* and *Anthoxanthum odoratum,* would be expected to make up the majority of viable seed which is incorporated into the hay. It is to be expected therefore that all the species found within a meadow would not be available to be fed to the stock and become incorporated into farmyard manure within any particular year.

Table 2.6 The start and finish dates of hay-making in two decades at Bowberhead Farm. The hay making starts when the first field is cut and finishes when the hay from the last field cut is stored in the barn. Therefore the date of the last hay cut is likely to be about a week earlier.

	1950's		1970's	
	Mean Date	S.E. (days)	Mean Date	S.E. (days)
Start	1st June	2.6	29th June	3.4
Finish	3rd August	5.3	21st July	2.7

It is possible that the straw bought into the farms in the Pennines for use as bedding material may also contribute seed to farmyard manure. Duchon (1948 in Simpson and Jefferson 1996) suggested that 25% of weed seeds in manure could emanate from straw although given modern arable weed levels this source of viable seed may be expected to be quite low. It would also seem unlikely that any arable weed species once dispersed into meadow vegetation would find suitable niches for germination and establishment.

Seed in the hay bales fed to stock can enter farmyard manure by two routes either directly through dropping from feed racks onto the barn floor or following ingestion by the stock. The dispersal of seed by animals following ingestion (endozoochory) has been widely reported. The classical examples usually involve fleshy fruits or berries. Other examples have involved the long distance dispersal of seeds by migratory birds (Clausen *et al.,* 2002). However it has been shown that herbaceous plants can also be spread by this method (Bulow-Olsen 1980; Welch 1985; Gardener *et al.,* 1993; Mt Pleasant and Schlather 1994; Andrews 1995; Malo and Suarez 1995a; Malo and Suarez 1995b; Ghassali *et al.,* 1998; Pakeman *et al.,* 1998; Rupende *et al.,* 1998; Pakeman *et al.,* 1999; Woldu and Saleem 2000). Jones, Noguchi and Bunch (1991) found up to a mean of 20.5 germinable seeds g^{-1} of dry faecal matter in cattle grazing legume-grass pastures in Queensland, Australia.

It is via this type of dispersal that sheep and cattle moving between planted pastures and natural vegetation have spread the African grass *Eragrostis lehmanniana* in the

USA (Anable, McClaren and Ruyle 1992; Fredrickson *et al.,* 1997), the European grasses *Holcus lanatus* and *Poa annua* onto islands of the Tristan da Cunha group in the South Atlantic (Wace 1967) and leguminous forbs into the tropical grasslands of Australia (Gardener, McIvor and Jansen 1993). Milton *et al.,* (1995) also showed the germination of the Australian saltbush seeds (*Atriplex lindleyi*) from the dung of sheep. Whilst Woldu and Saleem (2000) suggested that livestock play a major role in maintaining the biodiversity of grassland vegetation by the dispersal of readily germinating seed in East African grassland. More than 60 species were found in dung collected from cattle over a year.

Indeed it has been suggested that this process could be used as a tool to increase floristic diversity, by grazing animals in areas of high botanical diversity whilst viable seed is available for ingestion. The animals could then be moved to areas of less botanical interest when the seeds would then be egested and either germinate immediately (Bulow-Olsen 1980) or become incorporated into the soil seed bank and germinate at a later date. Endozoochorous dispersal has also been suggested as a method of spreading more productive pasture species over inaccessible ground (Gardener *et al.,* 1993, Ghassali *et al.,* 1998). For example Ghassali *et al.,* (1998) found that 58-72% of small seeded legumes survive passage through the digestive system of sheep in northern Syria.

Janzen (1984) speculated on the evolutionary role of the foliage of commonly grazed herbaceous species suggesting that it could attract herbivores to consume the seeds and subsequently aid dispersal over larger distances. Ridley (1930) found a total of 124 species of seed in the dung of cattle in Sweden and proposed that wild cattle, which occurred often in great numbers throughout the northern temperate regions, were responsible for the spread of many herbaceous species. He went on to state that domestic cattle may now play a part in the dispersal of herbaceous plants.

Whilst many species exhibit morphological characteristics presumed to enhance dispersal, large numbers of species lack any evidence of dispersal mechanisms (Ridley 1930; Hodgson *et al.,* 1995; Cain Damman and Muir 1998). Many have small hard seeds that are consumed by herbivorous vertebrates, but whether this constitutes an 'evolutionary design' can be debated (Janzen 1984; Collins and Uno

1985; Dinnerstein 1989). However differences in seed morphology may result in differential seed survival rates following the ruminant digestive process so it is possible that some of the species found in hay meadows may be favoured by endozoochorous dispersal to a greater extent than others.

Pakeman *et al.*, (1998) in a acidic grassland in southern England found that 37 species were dispersed by herbivorous rabbits. Whilst Gardiner *et al.*, (1993) demonstrated that 10 species of legume survived digestion by cattle better than 8 species of grass in a study of tropical and subtropical Australian pastures. There were however marked differences between species of legume and grass, with 0-64% of grass seeds surviving passage and 0-78% of legumes surviving passage. Indeed Malo and Suarez (1995b) found that the herbaceous legume *Biserrula pelecinus* found in Mediterranean pastures was increased in germinative capacity by between 5% and 280% following digestion by cattle.

Work on the comparative ecology of 458 species of plant in the UK by Grime, Hodgson and Hunt (1988) reported only 8 species as being dispersed by endozoochory, these 8 species were all plants in which the seeds are encased in fleshy fruits or berries. None of these species are however likely to occur in meadow vegetation. However notable in the list were 154 species for which no form of dispersal mechanism was given. These species were plants with small seeds (<0.1 mg) which have been thought to be capable of unassisted dispersal.

Welch (1985) demonstrated that 88 species found in Scottish upland pastures were dispersed by ruminant animals (sheep and cattle) but only a small number of species were frequently dispersed; these were *Agrostis capillaris, Calluna vulgaris, Juncus spp., Poa pratensis, Rumex acetosella* and *Sagina procumbens.* Species which gained greater cover on dung than in the existing vegetation were *Lolium perenne, Poa pratensis* and *Poa annua, Cerastium holosteoides, Rumex acetosella* and *Rumex acetosa, Stellaria* spp. and *Veronica* spp. Much or all of this cover arose from germination rather than vegetative colonisation. All of these are species that could be found in Pennine hay meadow vegetation however data on the number of germinating seeds in a given weight of dung were not presented.

Pakeman *et al.*, (2002) following a survey of herbivore dispersal (by rabbits or sheep) in a study of 10 differing grasslands in the UK concluded that a substantial quantity and range of species are dispersed by herbivores and that this could have significant effects on community dynamics and species richness. In this study sheep dung contained mean seed densities of 0.172-0.607 seeds g^{-1}.

The species which most commonly germinated from dung in this study were 3 species of *Juncus* and a number of dicotyledonous species including the genera *Cardamine, Cerastium, Sagina, Stellaria* and *Veronica* in addition grasses of the genus *Poa* were also commonly dispersed.

Simao Neto *et al.*, (1987) concluded that it was the rate of passage that was critical to the recovery of viable seed following ruminant digestion of tropical pasture plants. For the 6 species, percentage germination was between 28.6 and 77.8 after 24 hours whereas following 5 days between 0 and 32.3% were viable. Kaske and Englehardt (1990) showed that smaller particles pass through the digestive systems of sheep quicker, however Simao Neto *et al.*, (1987) found that no single physical seed characteristic could be correlated with the rate of passage through the ruminant digestive system. Pakeman *et al.*, (2002) in the survey of a selection of grasslands in the UK found that smaller, round seeds were more likely to be dispersed in this manner than larger elongated seeds. It was also noted that species which form a persistent seed bank were more resistant to digestion. It is possible that the adaptations required to survive ingestion are similar to those needed to survive in the soil seed bank. Evolution for one trait may have pre-adapted species for the other. Indeed, seed dormancy of some species of UK meadow plants mean some seeds fail to establish when sown in restoration schemes (Hopkins *et al.*, 1999); it is possible that manure storage or digestion could improve this germination rate.

Therefore in the context of northern hay meadows it is known that some species found in the meadows are capable of dispersal via the dung of cattle. There is however only limited information available regarding which species of plant has seeds that are capable of remaining viable in any number following the passage through the digestive system of ruminant animals. It is also not possible to make accurate predictions about which species may be more likely to be dispersed via this route. The passage through the digestive system is however not the only means by which viable seed can enter farmyard manure. A certain proportion of seed will fall directly from feed racks into the bedding material thereby not being affected by the digestive process.

Once viable seed has been incorporated into farmyard manure it must remain viable for the time that it is stored in order for it to be able to germinate, or become incorporated into the soil seed bank once spread onto the meadow. During storage within the manure heap seeds will be subjected to high temperatures which can denature enzymes, whilst uric acid and ammonia can cause seeds to rot. The temperatures found in manure heaps are also at an optimum for the growth of micro organisms which can cause rapid decomposition of seeds (Poincelot 1975).

The viability of seeds in manure has been shown to decrease over time in a number of studies (Rupende *et al.,* 1998; Remosova 2000). Rupende *et al.* (1998) showed that the average number viable seeds in manure from African grasslands fell by 60% and nearly 100% following storage for 2 and 5 months respectively. Some species showed increases in germination following two months storage. A few of the less common species were largely unaffected by storage. Remosova (2000) showed that with seeds of Czech grasslands losses of viability were lower after 1-3 months storage than after 4-6 months. Following 6 months of storage only 0-10% of seeds remained viable.

During the storage of manure in the middens it is possible that seed from surrounding vegetation may be dispersed by means such as wind onto the manure heap and then be spread onto meadows at a later date. Such seed could be of hay meadow plants or other types of plant. The prodigious dispersal of *Chamerion angustifolium* by wind (Thompson, Bakker and Bekker 1997) for example often leads it to colonise waste ground and it can often be seen growing on abandoned manure heaps.

2.4 Soil Seed Bank

Once spread onto hay meadows any viable seed within manure will be able to either germinate or become incorporated into the soil seed bank. Buried viable seed banks are of fundamental importance in the understanding of plant communities; they play a key role in conservation and restoration (Bakker 1989) and in making predictions of community responses to changing land use and climate (Hodgson and Grime 1990). Darwin (1859) in the Origin of the Species commented on the large number of seeds found to be present in only small quantities of soil.

Generalisation on the characteristics of seeds in relation to the soil seed banks were made by Harper (1977). He suggested that long term persistent seeds are characteristic of disturbed habitats, are usually annual or biennial and are often smaller in size. The relatively undisturbed nature of hay meadows and the dominance of perennials in the vegetation therefore points towards a persistent seed bank which does not contain all the species found within the meadows.

2.4.1 Seed Longevity

Circumstantial evidence for seed longevity in the soil has been shown at Rothamstead. An ungrazed meadow established on a site contained an equivalent to 320 arable weed seeds m^{-2} 58 years after any ploughing had taken place. Whilst Odum (1965) recorded one seed of *Chenopodium album* and three of *Spergula arvensis* germinating from a soil sample dated AD200 in Denmark.

Direct evidence for longevity of seeds in the soil can only be established from burial experiments. However due to the time consuming nature of these experiments few species have been studied in this way (McDonald *et al.,* 1996). Bekker *et al.,* (1998) buried 17 fen meadows species and found that following 1 year germination was significantly lower and after 2 years another significant drop in germination occurred. A few species of sedge showed significant increases in germination however.

Soil seed banks are often classified according to the methods described by Thompson and Grime (1979). This scheme was proposed on the basis of sampling of seed banks over a period of one year. Seeds are classified firstly as either persistent, seeds found in the soil throughout the year, or transient, seeds only found in the soil in certain times of year. Species with transient seed banks are then further divided into those with seeds only present in summer (Type I), only in winter (Type II). Persistent seed banks are divided into with (Type III) or without (Type IV) a pronounced seasonal peak.

Thompson, Bakker and Bekker (1997) however pointed out that despite the usefulness of this classification it was deficient in certain areas. Namely:

1. In most studies the detailed knowledge of seasonal dynamics necessary to separate the types is not available
2. Types III and IV are ends of a continuum and there is also evidence that the same species may behave differently in different conditions and in different places.
3. The seed bank type gives very little information on the longevity of seeds in the soil and this information is important in understanding how changes to management will affect plant communities.

In order to overcome these problems Thompson, Bakker and Bekker (1997) proposed the adoption of the following classification

1. Transient. Species with seeds which persist for less than one year
2. Short-term persistent. Species with seed present in the seed bank for more than one year but less than five years.
3. Long term persistent. Species with seed that persist in the soil for at least five years.

Clearly some species are capable of remaining in soil and germinating many years later. If the composition of the community has changed over time, seeds from previous communities may persist in the soil long after the species have been lost from the vegetation (Thompson 2000).

A relationship between seed size and shape and persistence in the seed bank has been recognised (Thompson and Grime 1979). Persistent seed tend to be small and compact. These characteristics are also the same as those found by Pakeman *et al.*, (2002) to be associated with endozoochorous dispersal of in many UK grassland species.

Due to the interest in recreating species rich hay meadows, a great deal of research has been carried out on the seed banks of hay meadows as they are seen as potential sources of viable seed in hay meadow restoration. However even in plant communities with a long history of stable species composition many species present in the vegetation may be absent from the soil seed bank. For many perennial species, significant differences occur between the species composition of the soil seed bank and the vegetation found in the above ground part of the plant community.

In pasture communities of a valley in the Picos de Europa mountains of Spain Lopez-Marino *et al.* (2000) found 119 species of plant in the vegetation and 104 species of seed in the soil, however only 54 of these species were common to both. Whilst in a study of seed bank progression over a period of 25 years in a Nature reserve in the Netherlands Bekker *et al.* (2000) showed, that following the re-establishment of traditional management practices the soil seed bank is unlikely to determine the hayfield succession. This was because the composition of the soil seed bank tended to follow that of the above ground vegetation rather than direct it.

In the UK, a study of the seed bank of an *Alopecurus pratensis-Sanguisorba officinalis* (MG4) species rich flood meadow in Oxfordshire showed that the majority of species present were of the transient or short term persistent seed bank category (McDonald *et al.*, 1996). At total of 3376 seeds m^{-2} were recorded in the 0-10 cm soil cores. Despite being a dominant part of the vegetation the perennial herb *S. officinalis* was found to be not present in the seed bank. Within Pennine hay meadows the species composition of soil seed banks were found to be similar in species composition to those of improved meadow vegetation rather than the vegetation from which they are found (Smith *et al.*, 2002).

The absence from the seed bank of a number of the perennial species abundant in the vegetation, due to a lack of persistence (Chambers and MacMahon 1994) or the reliance of such species on vegetative reproduction means the main goal of restoration management is therefore to ensure an influx of seeds of desired species into a site.

The application of manure from a traditionally managed sward could provide a rich source of seed for incorporation into the soil seed bank. Jones, Noguchi and Bunch (1991) found that cattle dung deposition significantly increased the quantities of seed in the soil seed bank of pastures in Queensland, Australia. Seed numbers in the soil increased from 6 760 seeds m^{-2} in lightly stocked paddocks to 45 480 seeds m^{-2} in heavily stocked paddocks. Whilst Dai (2000) found that cattle dung deposition could change the relative abundance of species in the soil of limestone grassland in Sweden. One such species was the grass *Anthoxanthum odoratum* which is common in Pennine hay meadows. This species increased in frequency from 6.7% to 20.0% in soil under dung patches. However, Smith *et al.*, (2002) found no relationship between manure application and the species present in the soil seed bank. This study was however carried out on a meadow subject to restoration management and the source of the manure was not from a traditionally managed species rich sward.

2.5 Seed Rain

Another source of propagules available for the colonisation of gaps created by farmyard manure is the seed rain. The seed rain is defined as propagules dispersing into an area (Harper 1977). Whilst the application of farmyard manure is proposed as contributing to this seed rain this is not the only source seed rain. Seed could disperse from adjacent vegetation. However a large source of seed within this system is likely to be produced during the period in which the meadows are 'shut-up' for the growth of the hay crop.

The collection with seed traps and subsequent identification of seeds contributing to the seed rain of grasslands has been carried out in a number of studies (Jeffereson and Usher 1989; Peart 1989; Schott and Hamburg 1997; Urbanska and Fattorini 2000). Whilst Jefferson and Usher (1989) reported that the seed rain of grasslands found within disused chalk quarries in Yorkshire were closely related to the species composition of the grassland, more so than the seed bank. However this is unlikely to be the case within meadows due to the restricted time frame for seed production compared to such grasslands.

Jensen (1998), in a study of abandoned species rich wet meadows in Germany, found that the seed rain in improved meadows subject to restoration management consisted almost entirely of species already present in the vegetation. No propagules of the desired meadow species were caught in seed traps. In total 75 200 seeds of 45 species were trapped but 3 species *Scirpus sylvaticum, Juncus effusus* and *Urtica dioica* accounted for 65% of all seeds. Seeds of herbaceous and woody species not present in the meadows were caught. It was concluded that seed rain of abandoned meadows has an impact on succession to woody scrub but not on reversion to meadow vegetation.

In the Pennines a number of early flowering species could be expected to produce ripe seed prior to the hay being cut. For example the annual species *Rhinanthus minor* which is common within such meadows forms a transient seed bank and relies each year on producing seed which germinates from the seed bank the subsequent spring (Grime, Hodgson and Hunt 1988). If this species is unable to set seed it will not be able to persist within the meadow through survival within the soil.

No study has been made of the seed rain prior to hay cut within Pennine meadows, however, in a study of seed shed during hay making in a traditionally managed Pennine hay meadow Smith, Shiel and Pullan (1996) showed that large quantities of seed were deposited onto the surface of the meadow. The simulated hay making process for each 21kg bale produced 436 300 seeds. Twenty pit fall traps with an aperture of $40.7cm^2$ were also placed within the meadow. During hay making a mean of 151 (± SE 45.0) seeds of the grass *Cynosurus cristatus* and 138 (± SE 28.0) of *R. minor* for example were recorded. In each experiment however no measure was made of seed viability. Comparison of these results with those produced by Wells, Frost and Bell (1986) suggested that the quantities of seeds within hay bales and the quantity dropping during hay making were roughly similar. In both cases the large majority of seeds were made up of grasses and herbs of lesser conservation interest.

2.6 Vegetative growth

Whilst the creation of gaps within grassland vegetation has been shown to be a key requirement for recruitment of species by seed (Bullock *et al.,* 1995), the vegetative spread of plants into gaps has also been shown to be important in grassland communities (Marriot *et al.,* 1997). Small patches of bare ground, which might be produced by manure application, have a higher ratio of edge as compared to patch area. This makes them more easily colonised by vegetative spread of plants (Arnthorsdottir 1994) than by seedling colonisation.

Clonal growth into gaps in grassland has been shown to account for between 59 and 95% of colonising ramets (Rusch and van der Maarel 1992, Arnthorsdottir 1994, Bullock *et al.,* 1995). The colonisation of vegetation gaps within species rich grasslands or hay meadows has not been studied. However, the high proportion of species capable of clonal spread makes it likely that this route for colonisation could be important as species show clear differences in their abilities to colonise gaps by vegetative means (Bullock *et al.,* 1995). Indeed, Schmid and Harper (1985) showed that perennial grassland species are particularly capable of fast clonal expansion into gaps.

2.7 Aims and Objectives

It is clear that a number of aspects of the management of Pennine hay meadows have been very well studied such as grazing, cutting and fertiliser application. However the dispersal of seed within the meadow system has been less well studied.

In particular the role that farmyard manure could play in the dispersal of hay meadow plants has not been investigated. Bakker *et al.*, (1996) point out the need for the study of "Dispersal and germination niches created by dispersal agents". The study of this avenue for dispersal necessitates a widening of the study of hay meadows. Rather than looking at the meadows in isolation this study will look at a process that occurs at the whole farm level.

The aims of this study are therefore to:

1. Determine the viable seed contents of hay, manure and dung taken from traditionally managed Pennine farms.
2. Determine how the viable seed content of manure changes with storage.
3. Establish how the soil seed bank from hay meadows receiving applications of farmyard manure is related to the viable seed content of the manure.
4. Investigate the vegetation that grows into gaps produced by manure applications and see how it compares to the viable seed content of manure and the soil seed bank.

3. The comparison of viable seed content of farmyard manure, hay and dung with meadow vegetation from which it originates.

3.1 Introduction

Traditional management of meadowland in the Pennine Dales involves the spreading of farmyard manure in order to maintain soil fertility. The farmyard manure spread on meadows in the spring is the soiled bedding material of the animals housed in barns over the winter. These animals have been fed the hay cut from the meadows the previous summer. As this feed is likely to contain large quantities of seed, opportunities for the dispersal of meadow plants seed arise. These seeds could pass into the manure via the digestive system of the animals or fall directly into the manure from the feed racks. The manure will then be stored for varying lengths of time before it is spread, along with any viable seed it contains, onto the meadows in the spring.

Hay from species rich swards has been shown to contain large quantities of viable seed (Wells *et al.,* 1986). This has led to the successful investigation of hay strewing as a practical means of increasing botanical diversity, through propagule introduction in species poor swards (Wells *et al.,* 1986, Jones *et al.,* 1995, Atkinson *at al* 1995,Mortimer *et al.,* 2002). However the hay used in these experiments which are often small scale, is "green" hay which is usually not dried or turned and so not hay in the commercial agricultural sense. The quantity and species composition of viable seed found in meadow hay will be dependent on a number of factors including the species composition of the meadow it is taken from, the maturity of the sward from which the hay is cut and the management of the hay cutting, turning and baling operations (Jones *et al.,* 1995; Smith *et al.,* 1996).

The quantity and species make-up of viable seed in the hay will in turn influence which species are ingested by the stock, or are able to fall directly onto the barn floor. The viability of seed of different species is known to be affected by ruminant digestion in different ways (Neto and Jones 1987; Neto *et al.*,., 1987; Blackshaw and Rode, 1991 and Ocumpaugh and Swakon, 1993). In the majority of cases a reduction in seed viability is bought about by the digestive system. The extent of this reduction can however vary between species (see Chapter 4). The type of feeding system and regularity of mucking out will also affect the quantity of seed that is able to become incorporated into farmyard manure.

It is also expected that the storage of manure will affect the viability of different species in different ways (Rupende *et al.*, 1998). Once viable seed has been incorporated into farmyard manure it must remain viable for the time that it is stored in order for it to be able to germinate once spread onto the meadow. The viability of seeds in manure has been shown to decrease over time in a number of studies (Rupende *et al.*, 1998 and Mt. Pleasant and Shlather 1994). However seed has been shown to remain viable in farmyard manure after five months storage in tropical conditions (Rupende *et al.*, 1998).

In order to asses the potential for farmyard manure to act as a dispersal agent of viable seed of hay meadow plants it is necessary to determine the quantity and species composition of that manure and how it changes over time.

The purpose of this experiment was therefore to determine:

1. The quantity and composition of viable seed in hay cut from two differing traditionally managed meadow systems.
2. The quantity and composition of viable seed in samples of dung collected from cattle fed exclusively on hay from the same two traditionally managed sources.
3. The quantity and composition of viable seed in fresh farmyard manure as well as manure from the same sources following storage for 3 months, 6 months and a year.

The overall hypothesis to be tested is therefore that farmyard manure is a possible medium for the dispersal of viable seed of hay meadow plants.

3.2 Methods

3.2.1 Estimating the characteristics of viable seed in meadow hay samples from two locations.

3.2.1.1 Hay Sample collection.

Ten bales of meadow hay were obtained from Piper Hole Meadows (NY 726 033). The hay cutting commenced on 5/7/99 and was completed by 12/7/99. Ten bales were also collected from New House Farm (SD 933 643). Here the hay cut commenced on 10/7/00 and was completed by 14/8/00. Samples of approximately 1 kg of hay were taken from 5 of the bales for each site and weighed. These samples were then sieved through 2 coarse sieves (75 mm and 25 mm mesh), in order to reduce the sample bulk and ensure all of the seeds were separated from flowering stalks.

3.2.1.2 Germination of seeds from hay samples.

These samples, which mainly consisted of dust, seeds and associated flower parts and stalks, were then spread in a thin layer (no more than 0.5 cm thick) onto 2 or 3 seed trays of size 35 cm by 25 cm containing sterile potting compost. The samples were kept well watered in an unheated glasshouse for a year; this was to enable any cold chilling requirements such as that shown by *Geranium sylvaticum* (Hill 2000) to take place. Germinating seedlings were identified, counted and removed weekly. Seedlings which needed to be grown on for identification were re-potted and grown separately. Due to the difficulty involved in identifying certain of the more common species were identified to a group. *Ranunculus repens*, *R. bulbosus* and *R. acris* were all recorded as *Ranunculus* sp. and *Trifolium repens* and *T. pratense* were recorded as *Trifolium* spp.

The length of time required to enable all of the viable seeds in a sample to germinate largely depends on the thickness of the layer spread on the sterile sub-soil (Thompson *et al.*, 1997). The thickness of the sample layer should ideally be less than 1cm. Ter Heerdt *et al.* (1996) showed that a soil sample that had been concentrated took 6 weeks for full germination to occur whereas similar non-concentrated samples took 4 to 6 months. It has been shown that only seeds at the surface will germinate (Williams 1969). Seeds that are buried more deeply may be unable to germinate because of the reduction in light intensity (Fenner 1985). In order to overcome this problem following periods in which no germination occurred the samples were thoroughly mixed.

3.2.2 Estimating the characteristics of viable seed in farmyard manure collected from two different traditional meadow systems.

3.2.2.1 Farmyard Manure Sample Collection and Storage.

Fresh farmyard manure was collected from barns at Piper Hole Meadows in the winter of 1999 and New House Farm in the winter of 2000. The stalls containing the cattle are mucked out daily at these farms and it was this manure which was obtained. 2 samples of approximately 1 kg were taken and weighed, whilst the remaining manure was stored outside, in large plastic tubs with a diameter of approximately 1.0 by 1.5m deep. The tubs were holed in the bottom to allow rainwater to flow through. 2 further samples were taken from the middle of the stored manure piles after 3 months, 6 months and a year.

3.2.2.2 Germination of seeds in samples of farmyard manure.

The 1 kg samples were sieved coarsely (75 mm and 25 mm mesh) with water, to reduce sample bulk. A fine 0.212 mm mesh sieve was used to collect the seeds and other fine particles. This size of mesh has been shown to be fine enough to catch seed of the majority of species including *Juncus* species, which have particularly small seeds (Thompson *et al.,* 1997). The material collected was then spread thinly (no more than 0.5 cm thick) onto seed trays, of size 35 x 21 cm containing sterile potting compost. The samples were kept well watered in an unheated glasshouse for a year. Germinating seedlings were identified, counted and removed weekly as in 3.2.1.2. Following periods in which no germination occurred the samples were thoroughly mixed.

3.2.3 Estimating the characteristics of viable seed in dung samples produced when housed cattle are fed hay from two different traditionally managed sources.

Farmyard manure is the soiled bedding material on which house animals are kept. One of its constituent parts is the animal dung which contains only material which has passed through the animal digestive system.

As described in 3.2.1 above ten bales of meadow hay were collected from Piper Hole and New House Farm. Once the samples to be analysed for viable seed content were taken the bales were split into approximately 1 kg samples for it to be fed to pairs of heifers. The hay was fed to the animals in 1 kg quantities at random from each bale in order to prevent the differences in individual bales skewing the results. The hay from each location was fed separately.

A pair of Limousin/Friesian cross heifers were housed separately for each run of the trial. In each case the heifers were fed a diet of hay from another source for several days prior to the commencement of the trial so that the digestive system was given time to adapt to a hay only diet. The animals were then fed the meadow hay for three days after which the stall was cleaned out and the dung discarded. Three days has been shown long enough for any seed from the previous hay diet to have cleared the digestive system (Simao Neto, Jones and Ratcliff 1987). The feeding of the meadow hay was then continued for days four, five and six with two dung samples collected at the end of the day the rest of the dung was removed and discarded a the end of each day. Approximately 1 kg samples were taken.

Once collected, the samples were weighed and sieved using the same method as the manure samples above. The samples were then spread thinly (no more than 0.5 cm thick) onto 1 or 2 seed trays 35 x 25 cm, containing sterile potting compost. The samples were kept well watered in an unheated glasshouse for a year. Germinating seedlings were counted, identified and removed weekly.

3.2.4 Assessment of the hay meadow vegetation.

3.2.4.1 Piper Hole Farm

During June 2000 the vegetation from four randomly placed 0.5 m x 0.5 m (0.25 m^2) quadrats in each of 6 meadows was identified to species and the percentage cover of each species estimated. The randomisation was restricted to vegetation found between 5m and 15m in from the edge of the meadow. This was done in order to avoid excessive trampling of the sward as it grew, thereby limiting damage to the crop while at the same time avoiding the botanical differences at the edge of the meadow which is often seen in this vegetation type.

3.2.4.2 New House Farm

The same methods were used within the 4 meadows at New House Farm except that 10 quadrats were used in the meadow known as Big Meadow.

3.2.5 Data Analysis.

The species composition of the meadow vegetation and the viable seed content of the hay, farmyard manure and dung samples were compared using Principal Component Analysis (PCA) plots of percentage occurrence, hay, manure and dung samples, with percentage cover data from the vegetation. The PCA ordination was completed using Canoco (version 4) (ter Braak and Smilauer 1998).

Canoco 4 is a computer program which enables the user to complete various types of ordination. Ordinations are used to infer relationships within large multi-variate data sets such as those found in the investigation of plant communities. The ordination diagrams graphically represent the community structure. The data used in this thesis do not involve the use of environmental data to explain the vegetation data. It is for this reason that an indirect gradient analysis is used. The use of PCA is advised if the analysis of data using Correspondance Analysis or Detrended Correspondence Analysis produces sample scores with less than 1.5 Standard Deviations as was the case here.

PCA is used to produce two plots. The sample plot shows a representation of the samples along two axis each sample is represented by a point. Those points grouped close together are more closely related in terms of species composition than points further apart. The species plot shows the same axis with arrows originating from the centre pointing towards the position on the two axis with which it is most strongly associated. The length of the arrow gives a representation of the strength of the relationship. Using this information it is possible to determine how closely related samples are and which species are most often associated with those samples.

3.3 Results

3.3.1 Piper Hole

3.3.1.1 Piper Hole meadow vegetation.

The species composition of the vegetation from which the hay samples for this experiment were cut is shown in Table 3.1. The constant species, those with a frequency of V or IV include a range of grass species as well as *Ranunculus acris, Rumex acetosa, Plantago lanceolata, Bellis perennis, Trifolium pratense, Myostis discolor, Cerastium fontanum and Taraxacum officinale agg..* Also included in these constant species are species of higher conservation value such as *Sanguisorba officinalis* and *Rhinanathus minor* whilst *Geranium sylvaticum* and *Filipendula ulmaria* also occur frequently.

Table 3.1 Frequency and abundance of species recorded in twenty four $0.5m^2$ quadrats from 6 Piper Hole meadows.

	Frequency V – I	Abundance (Domin value range)
Anthoxanthum odoratum	V	2-4
Holcus lanatus	V	1-6
Lolium perenne	V	1-4
Bromus hordeaceus	V	1-5
Plantago lanceolata	V	1-5
Rumex acetosa	V	1-4
Ranunculus acris	V	1-4
Bellis perennis	V	1-4
Cynosurus cristatus	IV	1-4
Poa trivialis	IV	1-4
Trifolium pratense	IV	1-5
Sanguisorba officinalis	IV	2-6
Rhinanthus minor	IV	1-4
Agrostis capillaris	IV	1-3
Myostis discolor	IV	1-3
Cerastium fontanum	IV	1-3
Taraxacum officinale agg.	IV	1-5
Anthriscus slyvestris	III	1-5
Filipendula ulmaria	III	1-5
Geranium sylvaticum	III	1-6
Phleum pratense	II	1-3
Poa annua	II	1-5
Festuca pratensis	II	1-2
Vicia sativa	II	1-4
Lathyrus pratensis	II	1
Alopecurus pratensis	II	1
Conopodium majus	II	1
Geranium pratense	I	2-5
Trifolium repens	I	1-4
Poa pratense	I	1
Dactylis glomerata	I	1
Arrhenatherum elatius	I	1-2
Ranunculus bulbosus	I	1-4
Alchemilla vulgaris agg.	I	1-3
Heracleum sphondylium	I	1-4
Stellaria media	I	1-3
Cirsium heterophyllum	I	2-4
Euphrasia nemorosa	I	1
Centaurea nigra	I	1-6
Caltha palustris	I	5

The number of species recorded in each of the quadrats sampled in the meadows at Piper Hole is shown in Table 3.2. A mean of 28 species were recorded in each quadrat from a total of 40 species. Appendix 1 shows the species found in each meadow at Piper Hole.

Table 3.2 The number of species in $0.5m^2$ quadrats from each of 4 quadrats per meadow recorded at PiperHole.

Number of Species							
	LK 1	**LK 2**	**LK 3**	**PH Field**	**FP**	**GB**	**Mean (±SE)**
A	16	19	26	17	15	16	
B	19	13	19	20	17	18	
C	16	14	20	18	19	18	
D	25	24	16	23	20	21	
Mean (±SE)	19(±2.1)	17.5(±2.5)	20.2(±2.1)	19.5(±1.3)	17.7(±1.1)	18.2(±1.0)	
Number of species	30	30	27	28	28	29	28
Total species							40

3.3.1.2 Quantity and composition of viable seed recovered from hay samples taken from Piper Hole Meadows.

The viable seed content of five 1 kg samples of hay bales cut from the Piper Hole meadows together with the mean (±SE) are given in Table 3.3 below.

Table 3.3 Total number of viable seeds kg^{-1} in hay samples taken from Piper Hole Meadows.

	1	**2**	**3**	**4**	**5**	**Total**	**Mean (±SE)**
Total viable seed kg^{-1}	1415	1668	2502	1142	1348		1615 (±237)
Number of species	28	19	23	24	23	36	23.4 (±1.44)
Total weight of hay bale (kg)	20.4	19.8	18.7	19.5	19.5		19.58 (±0.28)
Total viable seeds per bale.	28 866	33 026	46 787	22 269	26 286		31447 (±4215)

From Table 3.3 it can be seen that there was an average of 1615 viable seeds in each 1 kg sample of hay giving a mean of 31 447 viable seeds in each hay bale. Within the samples, an average of 23.4 species kg^{-1} was found and this level of species diversity was fairly consistent. In total, seeds of 36 different species were identified in the 5 samples. All the hay samples contained similar ranges of species, but as can be seen in Figures 3.6a and 3.6b one contained a different species make up. This was mainly shown in the number of *Urtica dioica* seeds and more *Holcus lanatus* seeds (see Appendix 2) than was seen in the other samples. This would suggest that this sample of hay may have originated from a locally eutrophicated patch within the meadow. Such patches are seen around feed troughs for example due to excessive dunging of the animals.

The numbers of viable seeds of each species in a 1kg sample of hay are shown in Table 3.4. In the table the species are listed in descending order of their prevalence, with the most frequently identified at the top of the list.

Table 3.4 The mean (±SE) number of viable seeds kg^{-1} and percentage frequency of each species found in hay samples from Piper Hole Meadows.

	Mean viable seed kg^{-1} (±SE)	Percentage frequency
Poa trivialis	845 (217)	100
Dactylis glomerata	144.8(72.4)	100
Lolium perenne	178.6(81.4)	100
Poa pratensis	117.1(33.2)	100
Agrostis capillaris	55.9(10.0)	100
Phleum pratensis	49.5(11.7)	100
Holcus lanatus	44.1(33.5)	100
Plantago lanceolata	38.9(24.0)	80
Myosotis discolor	18.(5.2)	100
Alopecurus pratensis	17.2(4.1)	100
Anthoxanthum odoratum	16.0(4.3)	100
Bromus hordeaceus	12.6(4.7)	80
Rumex acetosa	10.1(4.6)	100
Cerastium fontanum	10.8(2.7)	100
Bellis perennis	9.1(2.8)	100
Urtica dioica	7.6(7.6)	20
Ranunculus spp.	5.8(3.8)	60
Poa annua	4.4(2.7)	60
Cynosurus cristatus	4.4(3.1)	60
Polygonum persicaria	4.1(1.6)	60
Juncus effusus	4.0(1.7)	80
Montia fontanum	3.0(1.9)	40
Deschampsia cespitosa	2.7(2.4)	40
Arrehenatharum elatius	2.2(1.9)	40
Polygonum bistorta	2.2(0.3)	100
Juncus bufonius	1.7(1.1)	40
Agrostis stonlonifera	1.0(1.0)	20
Stellaria media	0.5(0.3)	40
Ajuga reptans	0.5(0.3)	40
Cirsium helenioides	0.4(0.4)	20
Vicia sepium	0.4(0.4)	20
Festuca pratensis	0.4(0.2)	40
Trifolium spp.	0.4(0.3)	40
Chamaerion angustifolium	0.3(0.3)	20
Festuca arundinacea	0.2(0.2)	20
Veronica chamaedrys	0.2(0.2)	20

It is clear that the grass *Poa trivialis* was easily the most prevalent species. The grasses *Dactylis glomerata, Lolium perenne* and *Poa pratensis* were also significant contributors to the total seed number. Only fourteen of the species were present at levels in excess of 10 seeds kg^{-1}.

Of the ten most prevalent species, eight were grasses and only two were broad leaved species, the perennial *P. lanceolata* and the annual *M. discolor*. Despite the hay samples coming from meadows containing many species of high nature conservation value such as *G. sylvaticum*, *S. officinalis* and *R. minor* the list above contains only one such species; *Cirsium helenoides* two viable seeds of which were recovered from just one of the samples. Otherwise there was no evidence of the hay containing species of high conservation value.

3.3.1.3 Quantity of viable seed recovered from samples of farmyard manure of various ages taken from Piper Hole Meadows.

Manure collected from the barns at Piper Hole was sampled fresh and after 3, 6 and 12 months of storage to establish the number and composition of viable seeds present. The data are presented as number of viable seeds kg^{-1} of manure.

Figure 3.1 shows the change with time in the number of viable seeds present as the manure aged. The mean number of viable seeds in fresh farmyard manure was 185 kg^{-1} and this remained relatively unaffected for three months of storage. However the number of viable seeds declined to a mean of 121 after 6 months and to just 24 after one year's storage.

Not only did the number of viable seeds decline over time, there was also a decline in the number of species present. This declined from a mean of 20 in fresh farmyard manure to a value of just 5 after one year of storage, with the major decline occurring between 3 and 6 months as was seen with the total viable seed content, see Figure 3.2. In total 27 different species were recorded in all the manure samples. In order to explore the significance of these results further the following sections examine the detail of the samples taken at each stage.

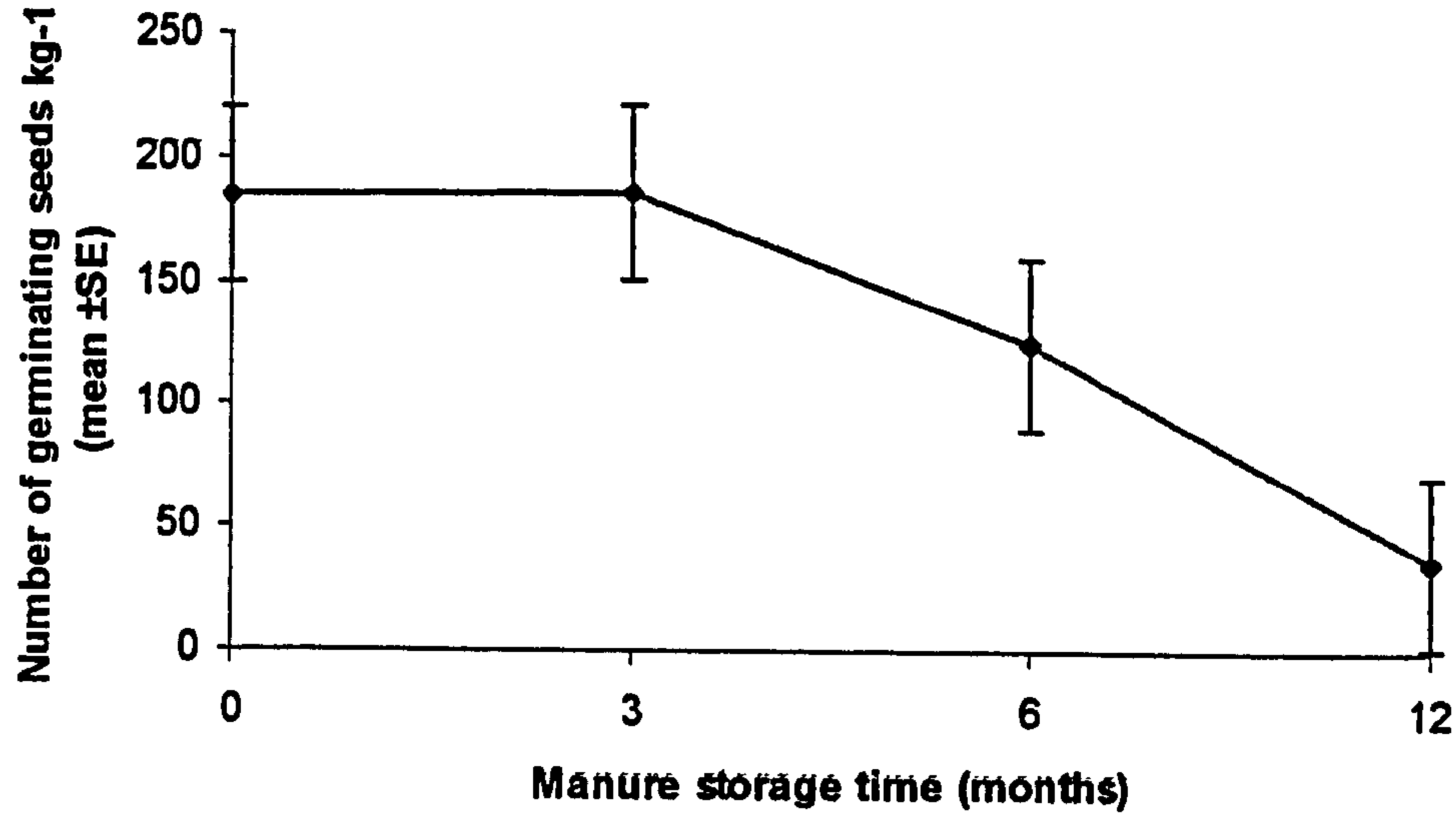


Figure 3.1 The effect of storage on the total viable seed content of farmyard manure from Piper Hole Meadows.

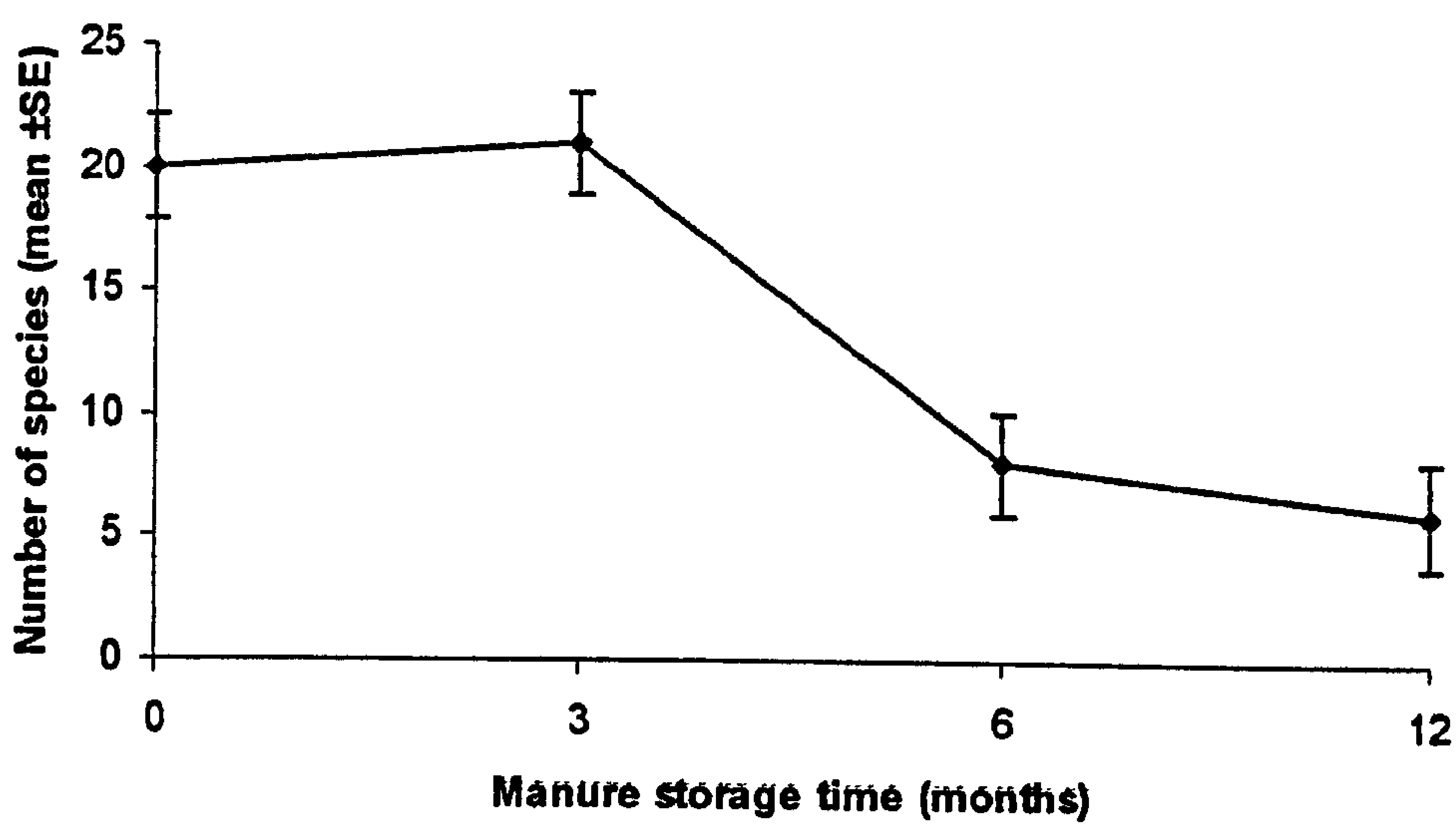


Figure 3.2 The effect of storage on the number of species found in farmyard manure from Piper Hole Meadows.

3.3.1.4 Quantity and composition of viable seed recovered from fresh manure samples taken from Piper Hole Meadows.

The mean number of seeds present in fresh farmyard manure was 185 seeds kg^{-1}. This is considerably less than the 1615 seeds kg^{-1} found in hay, although the two samples are not directly comparable (i.e. 1 kg of hay did not produce 1kg of farmyard manure). Examination of the number of species present revealed that fresh manure samples contained 15 species, rather less than the 23 in the hay from which the manure was derived.

Table 3.5 shows the species present in the manure of all ages, in terms of mean numbers of seeds kg^{-1}. As with hay samples *P. trivialis* was the most frequently detected species in the fresh manure. Some 70% of all seeds were of this species, reflecting an even greater dominance than in the hay. *L. perenne* continued to be present in significant amounts (11%) and indeed seven of the ten most prevalent species in fresh manure were also present in the 10 most common species in the hay. Almost 90% of all the seeds were accounted for by the five most common species all of which were present in reasonable quantities in the hay. Four of these species were grasses; the other was the dicotyledonous herb *M. discolor* which as a proportion of the total number, had become slightly more prominent in manure than hay. A list of the seed kg^{-1} in each manure sample is given in Appendix 3.

Table 3.5 The mean number of seeds kg^{-1} in fresh, three month old, 6 month old and 1 year old farmyard manure.

	Mean Seed kg^{-1} (± SE)				
Species	**Fresh**	**3 month old**	**6 month old**	**1 year old**	**Over all percentage frequency**
Poa trivialis	129.4(15.2)	130.7(8.6)	115.9(0.3)	19.1(0.0)	100
Lolium perenne	20.1(7.0)	20.7(10.4)	1.7(0.0)	0.9(0.0)	75
Poa pratensis	17.0(1.0)	6.5(5.9)	0.8(0.0)		50
Myosostis discolor	3.0(1.0)	1.3(0.08)			50
Dactylis glomerata	1.2(0.3)	2.1(0.4)			25
Anthoxanthum oderatum	0.9(0.9)	1.6(1.6)			25
Bromus hordeaceus	0.9(0.6)	1.6(1.0)			37.5
Plantago lanceolata	1.9(0.7)	2.0(0.8)		1.8(0.0)	62.5
Hordeum vulgare	1.5(0.8)				25
Juncus bufonious	1.4(1.4)	5.1(0.3)	1.5(0.0)	12.7(0.9)	75
Ranunculus spp.	0.6(0.03)	2.4(2.4)	0.9(0.0)		50
Juncus effusus	0.9(0.9)	0.3(0.3)			25
Cerastium fontanum	0.6(0.6)	0.6(0.6)			25
Phleum pratense	0.6(0.04)	0.7(0.7)			37.5
Agrostis capillaries	0.6(0.04)	0.9(0.9)			37.5
Festuca pratensis	0.6(0.6)				12.5
Trifolium spp.	0.3(0.3)	0.3(0.3)	1.3(0.5)		50
Poa annua	0.3(0.3)				12.5
Holcus lanatus	0.3(0.3)	0.6(0.6)			25
Rumex acetosa	0.3(0.3)	0.03(0.3)	1.5(0.0)	0.9(0.0)	50
Stellaria media		0.3(0.3)			12.5
Montia fontanum		0.3(0.3)			12.5
Cirsium arvensis		0.3(0.3)			12.5
Taraxacum officinlis agg.		0.3(0.3)			12.5
Chamaerion angustifolium			1.5(0.0)		12.5

3.3.1.5 Quantity and composition of viable seed recovered from manure samples taken from Piper Hole Meadows following 3 months storage.

In terms of number and species, samples stored for 3 months were similar to fresh samples. Thus Table 3.5 shows a dominance of *P. trivialis* and the same range of other species present. Compared to fresh samples *Ranunculus* species and the rush *Juncus bufonius* become more prominent.

3.3.1.6 Quantity and composition of viable seed recovered from manure samples taken from Piper Hole Meadows following 6 months storage.

Following 6 months of storage, seeds of only eight species germinated from the manure samples (Table 3.5). This represents a major fall from the 20 originally present in fresh samples. The number of viable *P. trivialis* seeds remained relatively unchanged at 116 kg^{-1} after storage, therefore the proportion this represents increased from 70% to 96%. Many of the grass species present in the hay and in fresh manure had ceased to be viable, with only 3 of the surviving eight species being grasses.

3.3.1.7 Quantity and composition of viable seed recovered from manure samples taken from Piper Hole Meadows following 12 months storage.

Following one years storage the viability of seeds in the manure was greatly reduced to only 24 viable seeds kg^{-1}. Only 5 species were present. Even *P. trivialis* which had previously remained relatively unaffected by storage was much reduced in terms of viability. Two of the five remaining species were grasses, with the rush *J. bufonius* becoming relatively more prevalent.

3.3.1.8 The changes in individual species numbers of Piper Hole Meadows farmyard manure following storage.

The quantity of viable seed kg^{-1} of *P. trivialis* only reduces to any great extent following 6 months storage (see Figure 3.2).

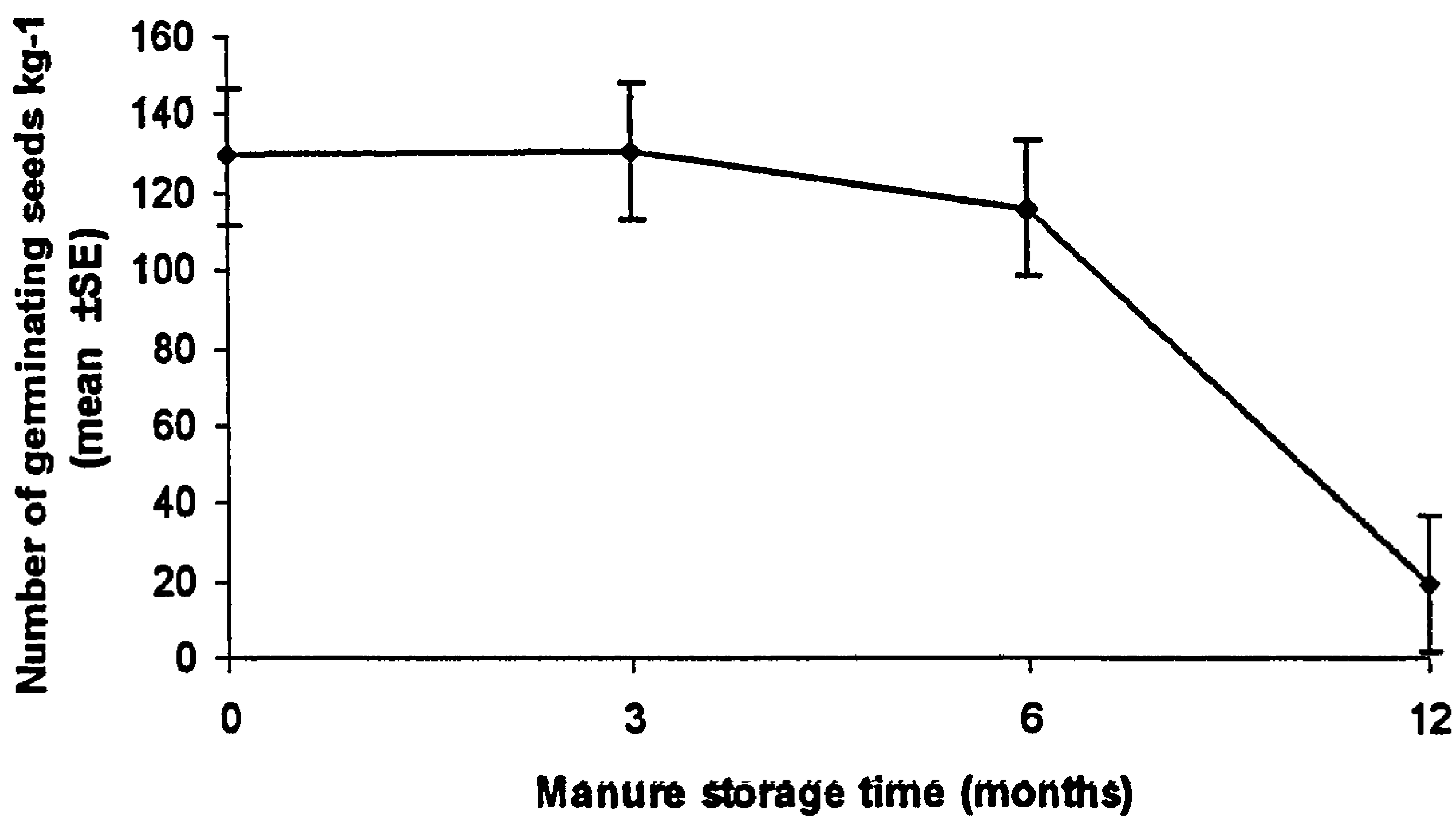


Figure 3.2 The effect of storage on the viability of *Poa trivialis* in farmyard manure from Piper Hole Meadows.

L. perenne shows a different pattern, the reduction in viability with this species occurs between three and six months storage (see Figure 3.3). The effect of storage on *Poa pratensis* however can be seen following just 3 months manure storage (see Figure 3.4).

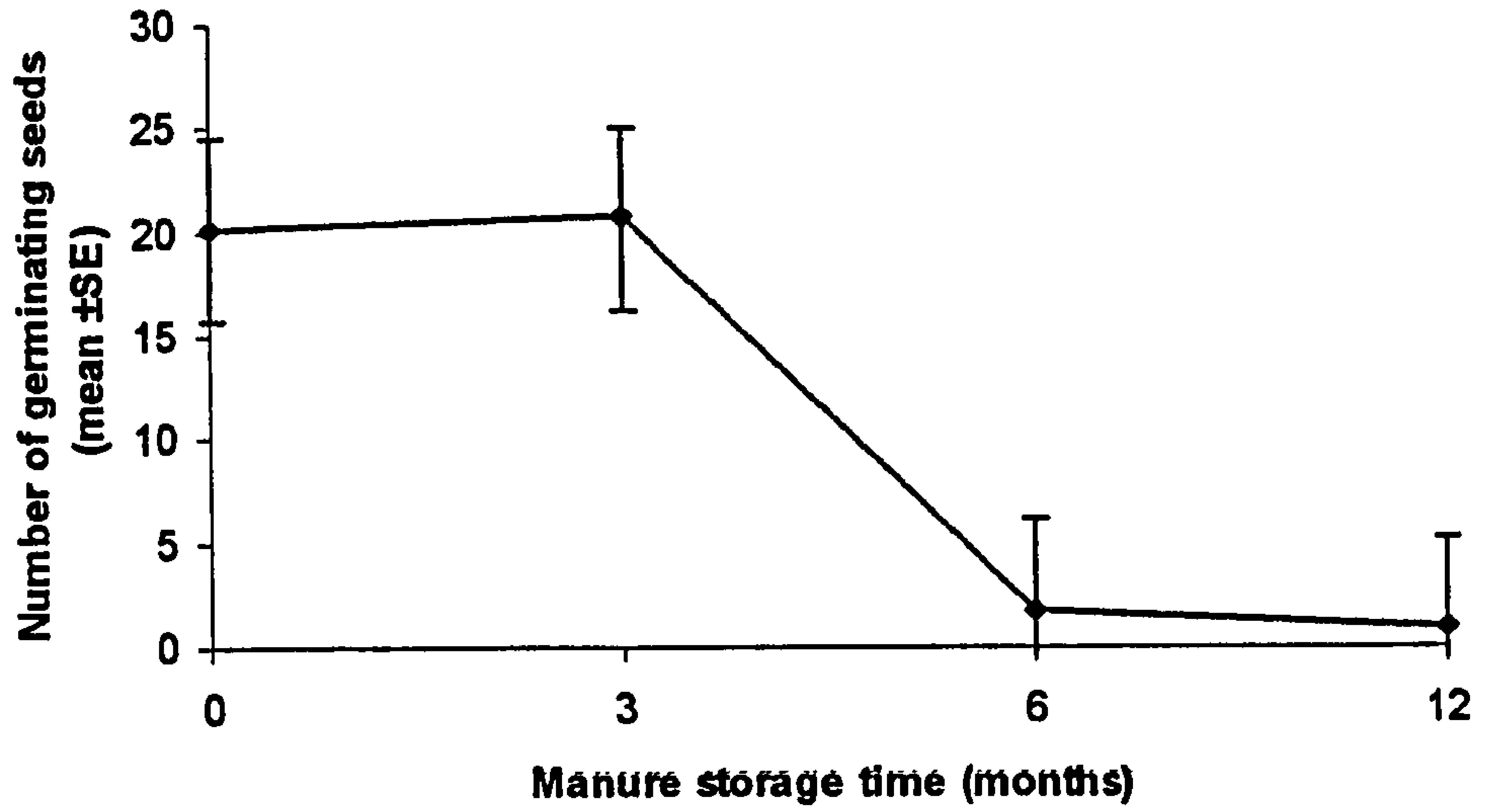


Figure 3.3 The effect of storage on the viability of *Lolium perenne* in farmyard manure from Piper Hole Meadows.

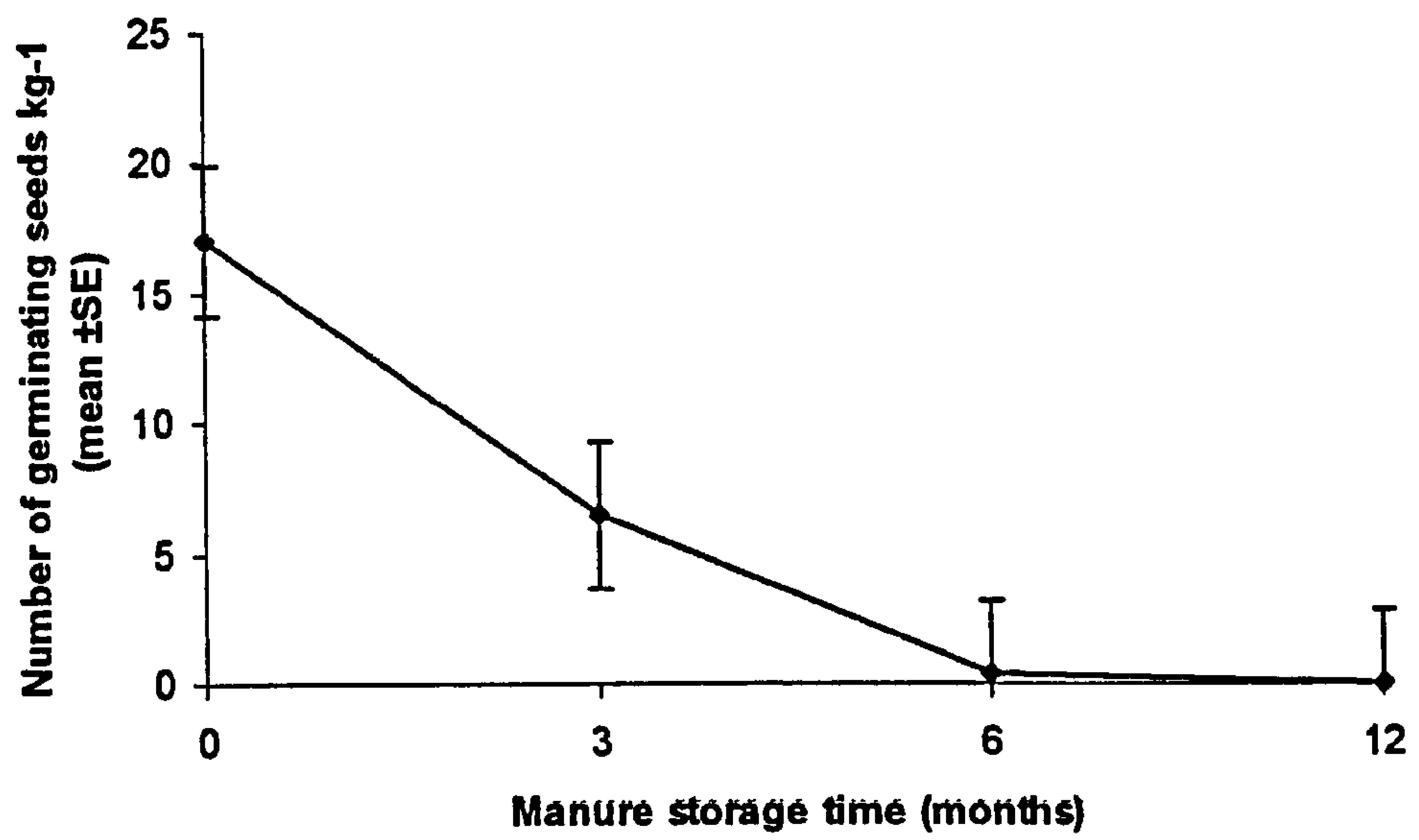


Figure 3.4 The effect of storage on the viability of *Poa pratensis* in farmyard manure from Piper Hole Meadows.

3.3.1.9 Quantity and composition of viable seed recovered from dung samples taken from cattle fed exclusively on hay from Piper Hole Meadows.

The number of viable seeds in a 1kg sample of dung contained a mean of 63 seeds kg^{-1}. This is much less than the 1615 viable seeds kg^{-1} which was found in the meadow hay fed to the animals, a reduction of 96.08 %. As can be seen in Table 3.6 a mean of 10 species were found in each kg^{-1} sample with a total of 16 species found in the entire set of 6 samples.

Table 3.6 The total number of viable seeds kg^{-1} and the number of different species kg^{-1} in dung samples taken from cows fed exclusively on hay from Piper Hole Meadows on three consecutive days.

	Day 1 Sample A	Day 1 Sample B	Day 2 Sample A	Day 2 Sample A	Day 3 Sample A	Day 3 Sample A	Mean (±SE)
Total viable seed kg^{-1}	46	45	66	69	99	54	63.28 (±8.2)
Number of species	10	11	10	10	11	10	10.33 (±0.2)
Total Number of species							16

As shown in Table 3.7 the dung samples were dominated by *P. trivialis* just as the hay and manure samples were. However of the 10 most common species in the dung samples only 4 were grasses, compared to the hay samples in which 8 of the most common species were grasses. In the dung samples the rushes *J. bufonius* and *J. effusus* were much more prominent as were the dicotyledonous species *Polygonum persicaria, Chamerion angustifolium and Trifolium spp.* From Appendix 4 it can be seen that the species make up of all the samples was very similar apart from Sample 1a which contained less *P. trivialis* and more *P. lanceolata* than the others.

Table 3.7 The mean (±SE) viable seeds kg^{-1} and percentage frequency of each species found in dung samples taken from cows fed exclusively on hay from Piper Hole Meadows.

Species	**Viable Seed kg^{-1}(±SE)**	**% Frequency**
Poa trivialis	37.39 (7.6)	100
Poa pratensis	6.15 (2.5)	100
Plantago lanceolata	4.21 (4.5)	100
Juncus bufonius	4.14 (1.8)	100
Juncu effusus	3.74 (1.4)	83
Lolium perenne	2.82 (1.6)	100
Agrostis capillaris	1.99 (0.7)	50
Polygonum persicaria	1.87 (0.7)	67
Chamerion angustifolium	1.31 (0.6)	33
Trifolium spp.	1.16 (0.4)	33
Cardamine flexuosa	1.05 (0.3)	17
Myosotis discolor	0.93 (0.4)	67
Rumex acetosa	0.84 (0.3)	67
Polygonum bistorta	0.78 (0.3)	33
Cerastium fontanum	0.69 (0.3)	50
Dactylis glomerata	0.60 (0.3)	33

3.3.1.10 Comparison of Piper Hole vegetation with hay, manure and dung samples.

From the PCA plot of samples, Figure 3.5a, it can be seen that the vegetation found in the Piper Hole meadows is extremely variable, a characteristic of unimproved grassland. Indeed Appendix 1 shows that the vegetation varies as much within each individual meadow as much as it does between the meadows.

The hay samples represented on the right of the diagram are all found below the manure samples with the fresh manure and 3 month old manure samples being closest to the hay sample in terms of species composition. The older manure samples and the half of the dung samples are generally found above the fresh manure sample indicating a change in species composition following digestion and manure storage.

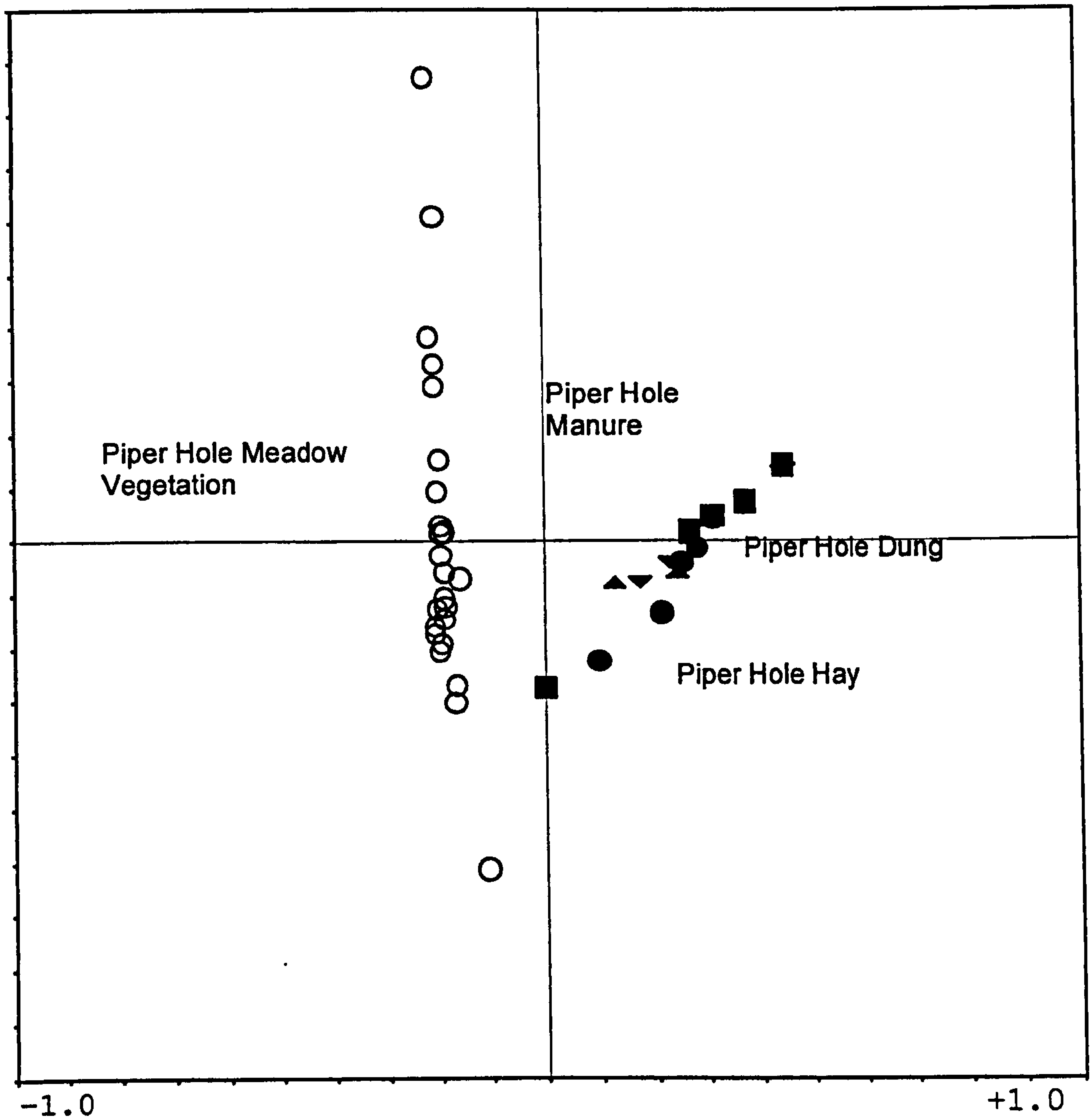


Figure 3.5a PCA bi-plot showing the site ordination of ○meadow vegetation, ●hay, ▲fresh manure, ▼3 month old manure, ►6 month old manure, ◄1 year old manure and ■dung samples from Piper Hole Meadows.

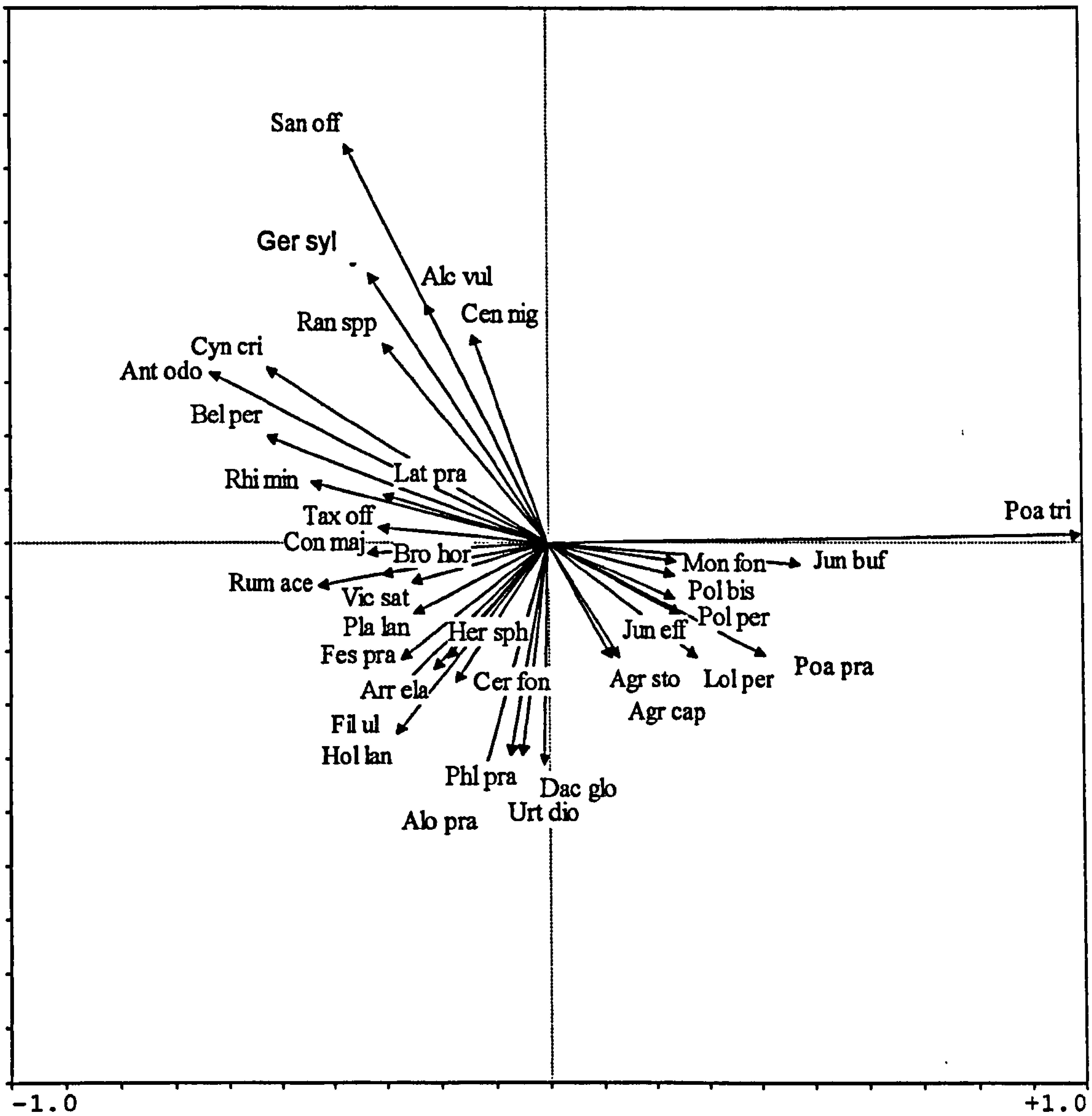


Figure 3.5b The PCA bi-plot showing the species ordination of meadow vegetation, hay, manure and dung samples from Piper Hole Meadows.

From the species plot (Figure 3.5b) it can be seen that *P. trivialis* is found in much larger quantities as seed in hay, manure and dung samples than would be expected given its percentage cover in the meadow vegetation whilst the rushes *J. effuses* and *J. bufonius* are characteristic of the hay, manure and dung samples and not the vegetation. The PCA plots show that the species found within the hay, manure and dung samples are not simply those found with the greatest frequency in the vegetation.

Table 3.8 demonstrates that a number of species such as the two rushes were recorded within the hay, manure and dung samples whilst not recorded at all within the meadow vegetation. The rushes may however still be found within the wetter flushes which are common in the meadows. The majority of the species found within the hay, manure and dung samples but not the vegetation could be expected to be found within the meadows, exceptions to this might be *Chamerion angustifolium* a wind dispersed species (Hodgson *et al.*, 1995) which may have blown into the meadow and become incorporated into the hay. *Hordeum vulgare* which was found within manure samples is the basis for the straw constituent of the manure.

The species of conservation value such as the perennial herbs *S. officinalis, G. sylvaticum* and *F. ulmaria* or the annual herb *R. minor* are all more frequently found in the vegetation than perennial grasses such as *Arrhenatherum elatius* and *D. glomerata* or the annual herb *Stellaria media* and yet they are not represented in the hay samples at all.

Table 3.8 The presence of species found in the meadows at Piper Hole in hay, manure and dung samples.

	Frequency In Vegetation	Occurrence In Hay Samples	Occurrence In Manure Samples	Occurrence in Dung Samples
Anthoxanthum odoratum	V	Yes	Yes	
Holcus lanatus	V	Yes	Yes	
Lolium perenne	V	Yes	Yes	Yes
Bromus hordeaceus	V	Yes	Yes	
Plantago lanceolata	V	Yes	Yes	Yes
Rumex acetosa	V	Yes	Yes	Yes
Ranunculus acris	V	Yes	Yes	
Bellis perennis	V	Yes		
Cynosurus cristatus	IV	Yes		
Poa trivialis	IV	Yes	Yes	Yes
Trifolium pratense	IV	Yes	Yes	Yes
Sanguisorba officinalis	IV			
Rhinanthus minor	IV			
Agrostis capillaris	IV	Yes	Yes	Yes
Myosotis discolor	IV	Yes	Yes	Yes
Cerastium fontanum	IV	Yes	Yes	Yes
Taraxacum officinale agg.	IV		Yes	
Anthriscus sylvestris	III			
Filipendula ulmaria	III			
Geranium sylvaticum	III			
Phleum pratense	II	Yes	Yes	
Poa annua	II	Yes	Yes	
Festuca pratensis	II	Yes	Yes	
Vicia sativa	II			
Lathyrus pratensis	II			
Alopecurus pratensis	II	Yes		
Conopodium majus	II			
Geranium pratense	I			
Trifolium repens	I	Yes	Yes	Yes
Poa pratense	I	Yes	Yes	Yes
Dactylis glomerata	I	Yes	Yes	
Arrhenatherum elatius	I	Yes		
Ranunculus bulbosus	I	Yes	Yes	
Alchemilla vulgaris agg.	I			

Heracleum sphondylium	I			
Stellaria media	I	Yes	Yes	
Cirsium heterophyllum	I			
Euphrasia nemorosa	I			
Centaurea nigra	I			
Caltha palustris	I			
Urtica dioica		Yes		
Juncus effusus		Yes	Yes	Yes
Polygonum persicaria		Yes		Yes
Montia fontanum		Yes	Yes	
Deschampsia cespitosa		Yes		
Polygonum bistorta		Yes		Yes
Juncus bufonius		Yes	Yes	Yes
Agrostis stolonifera		Yes		
Ajuga reptans		Yes		
Chamerion angustifolium		Yes	Yes	Yes
Festuca arundinacea		Yes		
Veronica chamedrys		Yes		
Hordeum vulgare			Yes	
Cirsium arvensis			Yes	
Cardamine flexuosa				Yes

3.3.1 New House Farm

3.3.2.1 New House Farm meadows vegetation.

The species composition of the vegetation from which the New House farm hay bales were cut is shown in Table 3.9. The constant species include a range of grasses including *Anthoxanthum oderatum*, *P. trivialis* and *Bromus hordeaceus* as well as the herbs *P. lanceolata*, *T. officinale agg.* and *R. minor*. *F. ulmaria* and *Alchemilla vulgaris agg.* are also frequent species.

Table 3.9 Frequency and abundance of species recorded in twenty five 0.5m^2 quadrats from 4 meadows at New House Farm.

Species	**Frequency V-I**	**Abundance Domin value range**
Anthoxanthum odoratum	V	3, 6
Trifolium pratense	V	4, 7
Bellis perennis	V	4, 7
Rumex acetosa	V	1, 8
Bromus hordeaceus	V	1, 7
Plantago lanceolata	IV	4, 8
Taraxacum officinale agg.	IV	4, 7
Rhinanthus minor	IV	1, 8
Cynosurus cristatus	IV	1, 4
Poa trivialis	IV	1, 4
Ranunculus acris	III	4, 7
Filipendula ulmaria	III	1, 6
Alchemilla vulgaris agg.	III	1, 5
Lolium perenne	III	1, 4
Alopecurus pratensis	III	1, 4
Cerastium fontanum	III	1, 4
Myosotis discolor	III	1
Holcus lanatus	II	1, 5
Ranunculus repens	II	1, 5
Anthriscus sylvestris	II	1, 5
Festuca rubra	II	1, 4
Phleum pratense	II	1, 4
Geranium sylvaticum	II	1, 4
Sanguisorba officinalis	II	1, 4
Ranunculus ficaria	II	1, 4
Conopodium majus	II	1, 4
Dactylis glomerata	II	1, 2
Stellaria media	II	1, 2
Veronica chamaedrys	II	1
Cirsium helenioides	I	6,7
Ranunculus bulbosus	I	1, 6
Carex nigra	I	1, 6
Juncus effusus	I	1, 5
Rumex obtusifolius	I	1, 5
Geum rivale	I	1, 4
Deschampsia cespitosa	I	1, 4
Carex panicea	I	1, 4
Caltha palustris	I	3
Heracleum sphondylium	I	1
Euphrasia nemorosa	I	1
Centaurea nigra	I	1
Cardamine pratensis	I	1
Poa annua	I	1
Festuca pratensis	I	1
Agrostis capillaris	I	1
Briza media	I	1

Table 3.10 shows the number of species recorded in each of the quadrats sampled within the meadows at New House Farm. A mean of 16.88 (±0.61) species were recorded within each quadrat from a total of 46 species. The full list of species within each quadrat is given in Appendix 5.

Table 3.10 The number of species recorded in each of the 0.5m^2 quadrats recorded in the New House Farm Meadows.

	Big Meadow	**Top Meadow**	**Middle Meadow**	**Bottom Meadow**	**Mean (±SE)**
A	22	16	19	17	
B	19	11	16	17	
C	16	13	15	13	
D	24	16	18	16	
E	19	15	16	12	
F	21				
G	20				
H	19				
I	16				
J	16				
Mean(±SE)	19.2(±0.9)	14.2(±1.0)	16.8(±0.7)	15.0(±1.1)	16.9(±0.6)
Number of species	37	26	27	24	28.5 (±2.9)
Total species					46

3.3.2.2 Quantity and composition of viable seed recovered from hay samples taken from New House Farm.

The number of viable seeds in five 1 kg samples from hay bales cut from the New House Farm meadows together with the mean (±SE) are given in Table 3.11 below. It is shown that there was an average of 375 (±132) viable seeds of hay in each 1 kg sample giving a mean of 8472 viable seeds in each hay bale. Within the samples, an average of 16.0 species kg^{-1} were found and this level of species diversity was fairly consistent as can be seen in Appendix 6.

Table 3.11 Total number of viable seeds kg^{-1} in hay samples taken from New House Farm.

	1	2	3	4	5	**Mean (±SE)**
Total viable seed kg^{-1}	399.1	137.7	104.4	840.0	393.1	375 (132)
Number of species	17	16	14	19	14	16(0.9)
Total Number of species						27
Total weight of hay bale (kg)	21.6	20.0	24.2	23.5	22.3	22.3 (0.7)
Viable seed per bale	8620	2754	2528	19698	8757	8472 (3116)

The species with viable seed found within the hay samples taken from New House Farm are shown in Table 3.12. *Bellis perennis, Plantago lanceolata* and *P. trivialis* were the most common species. The hay did not contain any of the species found within the vegetation from which the hay was cut that are considered to be of conservation value. The majority of the species found were grasses and annual herbs. In general these samples were more variable than those taken from Piper Hole hay.

Table 3.12 The mean viable seeds kg^{-1} of each species found in hay samples from New House Farm.

	Mean viable seed kg^{-1}(±SE)	Percentage Frequency
Bellis perennis	94.2 (46.2)	100
Plantago lanceolata	92.4 (33.4)	100
Poa trivialis	90.9 (23.5)	100
Lolium perenne	31.5 (13.6)	100
Bromus hordeaceus	23.5 (12.1)	80
Anthoxanthum oderatum	12.0 (5.2)	100
Myostis spp.	10.7 (4.0)	80
Trifolium spp.	5.1 (2.0)	100
Alopecurus pratense	2.0 (0.3)	100
Poa pratense	1.4 (0.5)	80
Cynosurus cristatus	1.2 (0.5)	60
Deschampsia cespitosa	1.2 (1.2)	20
Rumex acetosa	1.1 (0.3)	80
Ranunculus spp.	1.0 (0.3)	80
Cerastium fontanum	1.0 (0.5)	60
Juncus effusus	0.9 (0.6)	20
Holcus lanatus	0.8 0.6)	40
Phleum pratesne	0.7 (0.4)	40
Conopodium majus	0.6 (0.4)	40
Festuca rubra	0.5 (0.3)	40
Agrostis capilaris	0.5 (0.3)	40
Juncus bufonius	0.3 (0.3)	20
Cirsium arvense	0.3 (0.3)	20
Festuca pratense	0.3 (0.3)	20
Helictotrichon pubescens	0.3 (0.3)	20
Polygonum bistorta	0.3 (0.3)	20
Chamerion angustifolium	0.1 (0.2)	20

3.3.2.3 Quantity of viable seed recovered from samples of farmyard manure of various ages taken from New House Farm.

Samples of fresh farmyard manure taken from New House Farm contained a mean of 21.62 viable seeds kg^{-1}. Following three months storage samples were found to contain a mean of 22.98 viable seeds kg^{-1}. However despite the lack of a reduction in seed content following three months storage, a six months storage period produced a mean of only 5.14 viable seeds kg^{-1} and after a year of storage no viable seeds were found (Figure 3.6).

The hay samples contained a mean of 375 viable seeds kg^{-1} whereas the fresh manure samples contained only 21.62 viable seeds kg^{-1} the fresh manure samples the full list of species within New House manure samples is given in Appendix 7. This is a similar reduction to that seen with the Piper Hole samples. The mean viable seed kg^{-1} of manure at Piper Hole contained 8 % the viable seed content of the hay compared to a reduction of 6 % with the New House samples. At New House Farm the manure contained considerable less species than the hay samples; a mean of five (Figure 3.7) compared to a mean of 16 within the hay samples.

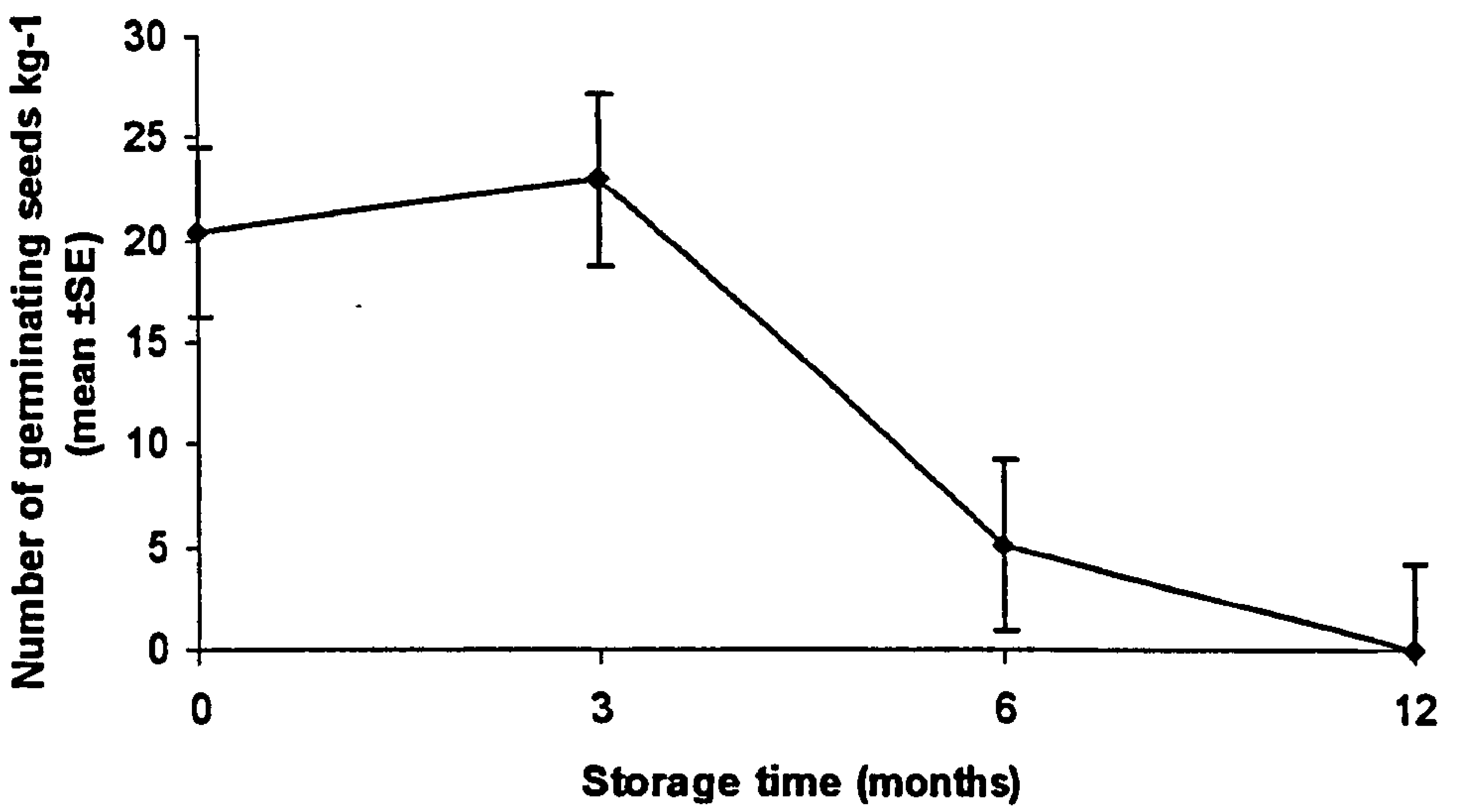


Figure 3.6 The effect of storage time on the total viable seed kg^{-1} of farmyard manure from New House Farm.

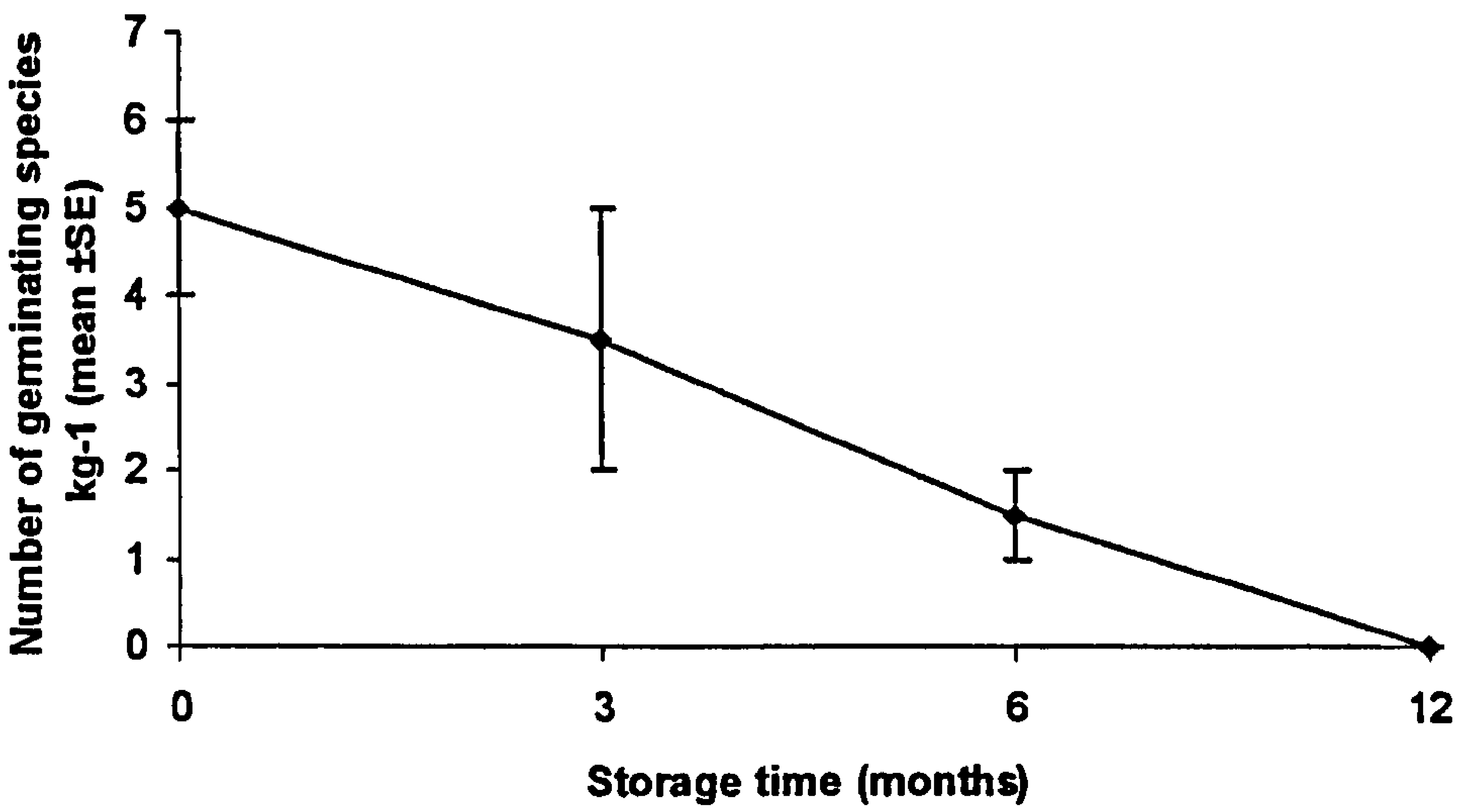


Figure 3.7 The effect of storage on the number of species kg^{-1} found in farmyard manure from New House Farm.

3.3.2.4 Quantity and composition of viable seed recovered from fresh manure samples taken from New House Farm.

Table 3.15 shows that whilst *B. perennis* was the most common species within the New House farm hay samples this was not the case with the fresh manure samples. *P. trivialis* was the most common species within the fresh manure samples. Indeed as well as *P. trivialis; P. lanceolata, L. perenne* and *J. bufonius* were all more common than *B. perennis*. The fresh manure samples, as with the hay samples, were however dominated by grasses and annual herb species.

Table 3.13.The mean viable seeds kg^{-1} and the proportion of each species found in farmyard manure samples of various ages taken from New House Farm

Species	Mean (±SE) viable seed kg^{-1}				
	Fresh	**Three Months**	**Six Months**	**One Year**	**Overall Percentage Frequency**
Poa trivialis	13.8 (6.7)	12.7 (1.3)	4.1 (1.0)		75
Plantago lanceolata	2.6 (1.3)	1.2 (1.1)			25
Lolium perenne	2.0 (0.6)				25
Juncus bufonius	2.0 (0.6)	0.6 (0.6)			25
Bellis perennis	1.4 (1.4)	1.1 (1.1)			25
Myosotis spp.	0.6 (0.6)	1.6 (1.6)			25
Anthoxanthum oderatum	0.6 (0.6)	4.0 (0.6)			37.5
Rumex acetosa		1.1 (1.1)			12.5
Urtica dioica		0.6 (0.6)			12.5
Juncus effusus			1.0 (1.0)		12.5

3.3.2.4 Quantity and composition of viable seed recovered from manure samples taken from New House Farm.

The manure which had been stored for three months was similar to the fresh manure in both species make up and quantity. Once again *P. trivialis* was the most frequent species with other grasses and annual herbs common. The New House manure which was stored for six months prior to germination contained only two species at very low numbers these were *P. trivialis* and *J. effusus*. Following one years storage the samples of manure from New House Farm failed to produce any viable seed at all.

3.3.2.5 The changes in individual species numbers of New House Farm farmyard manure following storage.

The very low numbers of seeds germinating within the manure from New House farm makes the investigation of individual species response to manure storage unreliable. The only species found in a high enough quantity was *P. trivialis*.

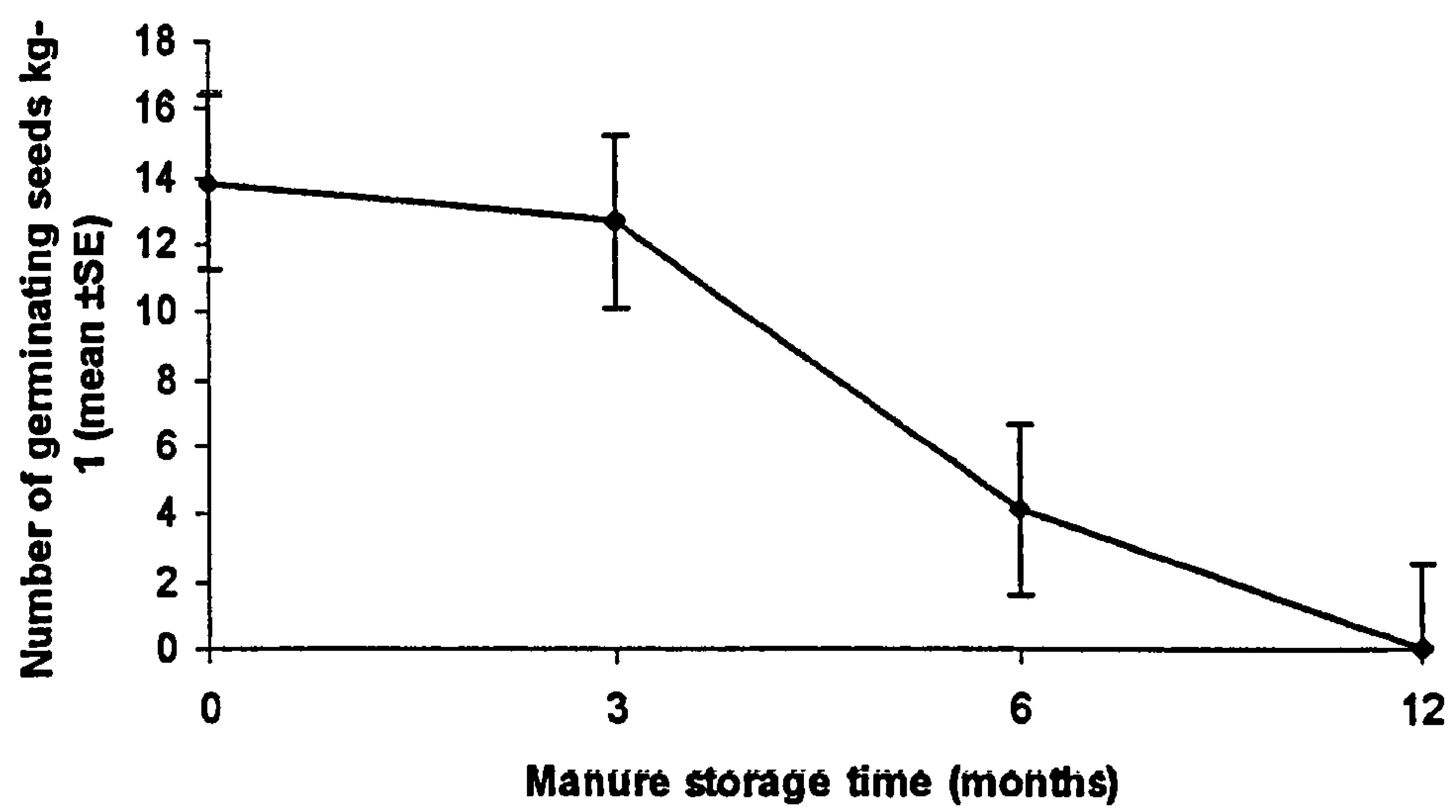


Figure 3.8 The number of germinating *Poa trivialis* seeds germinating following the storage of New House Farm manure.

The pattern of reduction of viable *P. trivialis* seed content of manure is similar to that found with *P. trivialis* seed in manure from Piper Hole, as shown in Figure 3.8. Little or no reduction is seen following 3 months storage, after 6 months storage the seed content has however dropped considerably. Following 12 months storage the seed content has dropped further still. This is a slightly different response to that seen in manure samples taken from Piper Hole which showed a reduction in germination only after 6 months storage.

3.3.2.6 Quantity and composition of viable seed recovered from dung samples taken from cattle fed exclusively on hay from New House Farm.

The quantity of viable seed and the number of species of viable seed found within dung samples of animals fed exclusively on hay from New House Farm is shown in Table 3.14. The means of 32.64 viable seeds kg^{-1} and 4.83 species kg^{-1} are considerably less than those seen in the hay samples and also less than those found in fresh farmyard manure. Appendix 8 shows the species composition of each dung sample.

Table 3.14 The total number of viable seeds kg^{-1} and the number of different species kg^{-1} in dung samples taken from cows fed exclusively on hay from New House Farm on three consecutive days.

	Day 1 Sample A	Day 1 Sample B	Day 2 Sample A	Day 2 Sample A	Day 3 Sample A	Day 3 Sample A	Mean(±SE)
Total viable seed kg^{-1}	18.7	45.6	36.6	23.0	36.0	35.4	32.6(4.0)
Number of species kg^{-1}	5	8	5	3	4	4	4.83(0.7)
Total Number of species							12

The species which were recorded from the dung samples are shown in Table 3.15. Once again the grass *P. trivialis* is the most prominent species. It is present in a larger quantity than *B. perennis* which was the most common species in the hay samples.

Two species of rush *J. bufonius* and *J. effusus* are also more conspicuous in the dung samples than they were within hay samples.

Table 3.15 The mean viable seeds kg^{-1} and the proportion of each species found in dung samples taken from cows fed exclusively on hay from New House Farm.

Species	Viable Seed kg^{-1}	Percentage Frequency
Poa trivialis	12.7 (2.3)	100
Plantago laceolata	4.7 (2.5)	50
Juncus effusus	4.0 (1.4)	66.66
Juncus bufonius	3.5 (2.5)	33.33
Bellis perennis	1.6 (1.2)	33.33
Lolium perenne	1.5 (0.8)	50
Trifolium pratense	1.3 (1.0)	33.33
Poa pratensis	1.1 (0.8)	33.33
Cynosurus cristatus	0.6 (0.3)	50
Bromus hordeaceus	0.6 (0.6)	16.67
Myosotis discolor	0.6 (0.6)	16.67
Cardamine pratense	0.2 (0.2)	16.67

3.3.2.7 Comparison of New House Farm meadow vegetation with hay, manure and dung samples.

The PCA plot in Figure 3.9a shows the meadow vegetation on the left. It is distinctly different from the hay, manure and dung samples on the right side of the plot. The meadow vegetation itself is somewhat variable.

The hay samples form a cluster of points showing their similarity to each other whereas the dung and manure samples, with the exception of two dung samples which form another cluster. The progression from fresh manure to older manure with an even larger dominance of *P. trivialis* is seen here as it was with the Piper Hole samples.

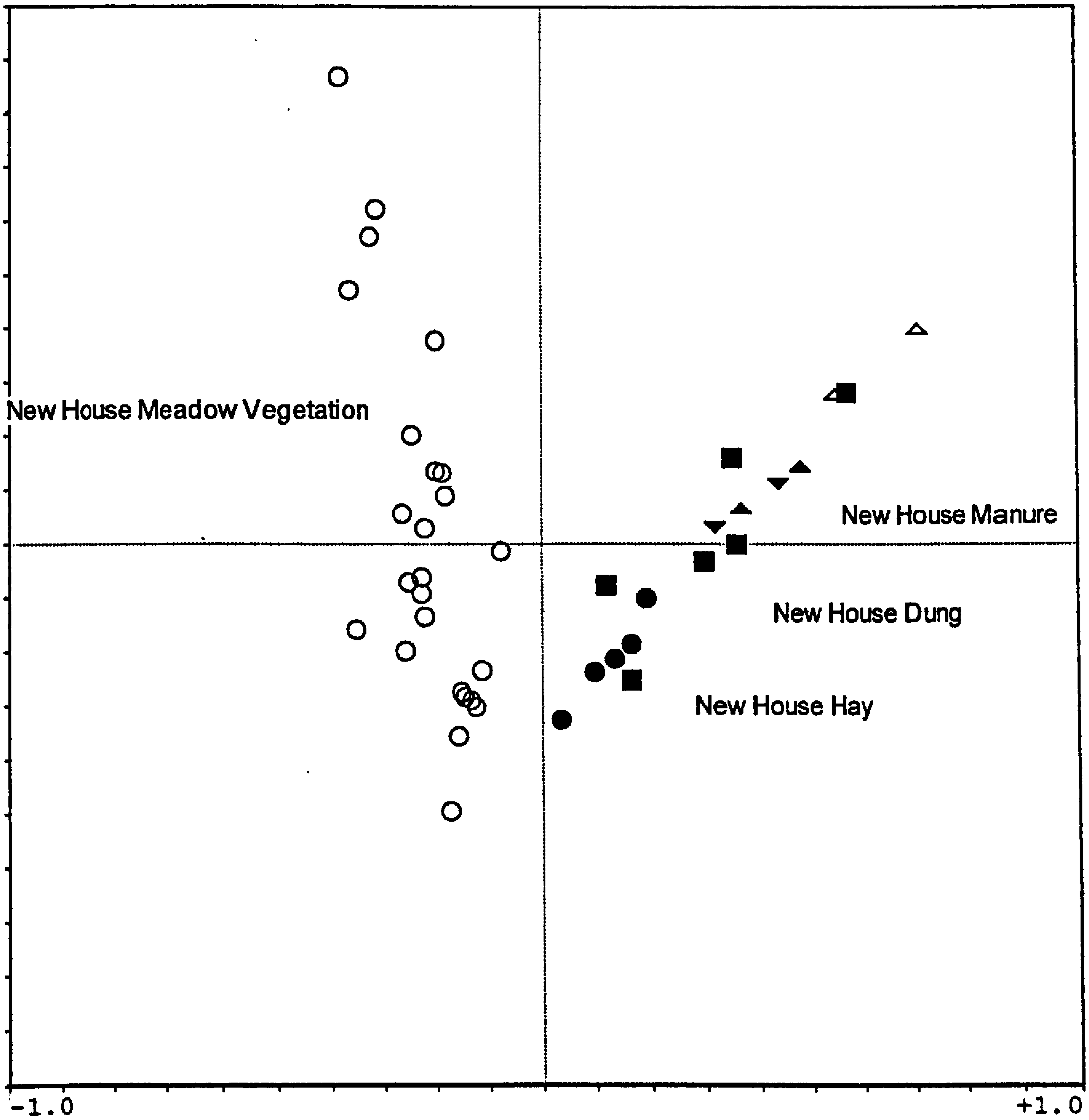
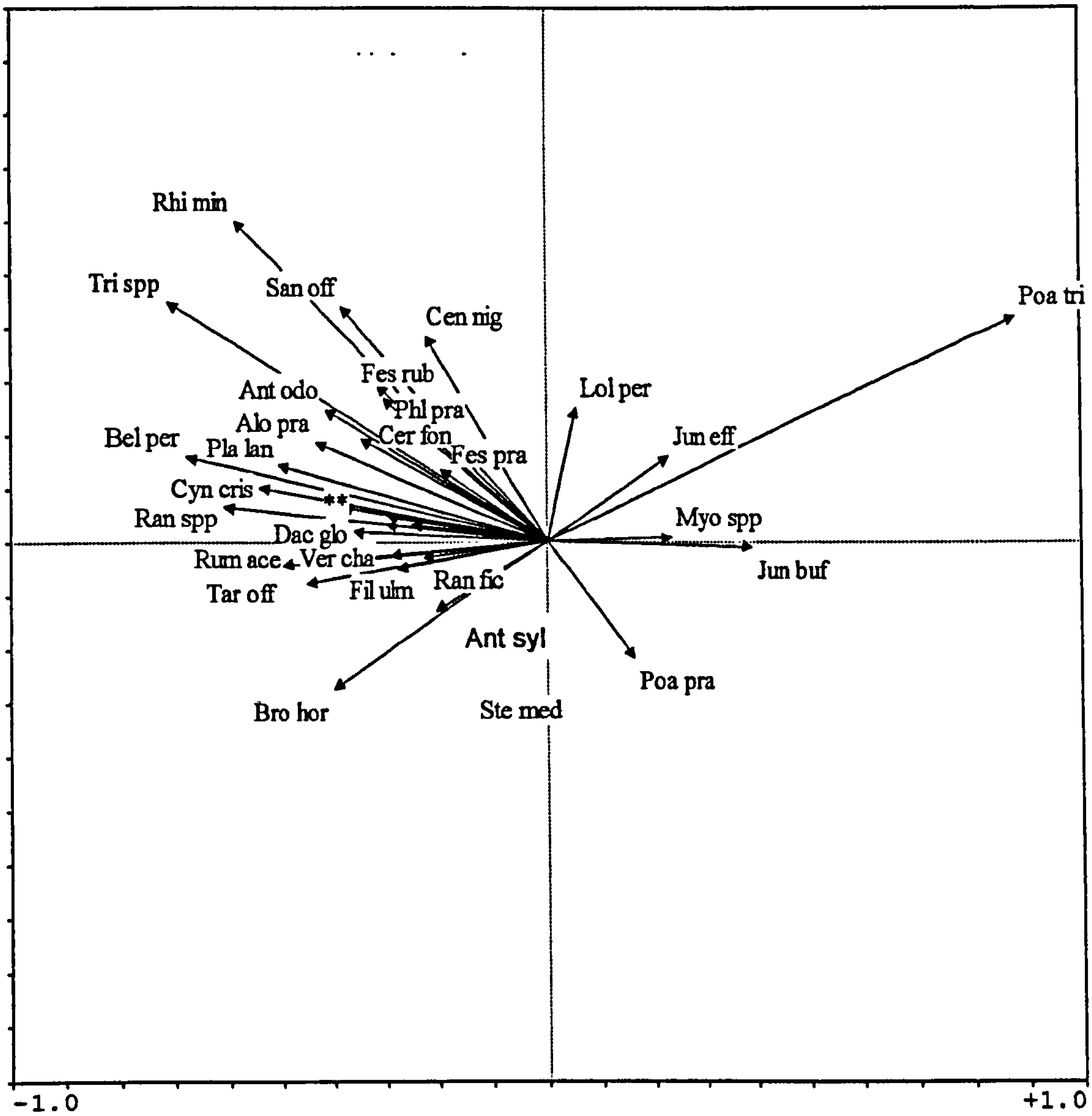


Figure 3.9a The PCA bi-plot showing the samples ordination of ○meadow vegetation, ●hay, ▲fresh manure, ▼3 month old manure, Δ 6 month old manure and ■dung samples from New House Farm.

Figure 3.9b shows the range of species on the left which are particularly characteristic of the vegetation rather than the hay sample and manure and dung sample clusters. The vegetation is typified by species such as *R. minor*, *G. sylvaticum*, *C. helenoides* and *Anthriscus sylvestris*. It is interesting to note that both *B. perennis* and *P. lanceolata* whilst being found in large quantities within the hay samples are found more often within the vegetation.



** *Con maj, Ger syl and Alc vul*

Figure 3.9b The PCA bi-plot showing the species ordination within New House Farm vegetation, hay, manure and dung sample

Table 3.16 The presence of species in meadow quadrats and hay, manure and dung samples from New House Farm.

	Frequency in vegetation	Occurrence in hay samples	Occurrence in manure samples	Occurrence in dung samples
Anthoxanthum odoratum	V	Yes	Yes	
Trifolium pratense	V	Yes		Yes
Bellis perennis	V	Yes	Yes	Yes
Rumex acetosa	V	Yes	Yes	
Bromus hordeaceus	V	Yes		Yes
Plantago lanceolata	IV	Yes	Yes	Yes
Taraxacum officinale agg.	IV			
Rhinanthus minor	IV			
Cynosurus cristatus	IV	Yes		Yes
Poa trivialis	IV	Yes	Yes	Yes
Ranunculus acris	III	Yes		
Filipendula ulmaria	III			
Alchemilla vulgaris agg.	III			
Lolium perenne	III	Yes	Yes	Yes
Alopecurus pratensis	III	Yes		
Cerastium fontanum	III	Yes		
Myostis discolour	III	Yes	Yes	Yes
Holcus lanatus	II	Yes		
Ranunculus repens	II			
Anthriscus sylvestris	II			
Festuca rubra	II	Yes		
Phleum pratense	II	Yes		
Geranium sylvaticum	II			
Sanguisorba officinalis	II			
Ranunculus ficaria	II			
Conopodium majus	II	Yes		
Dactylis glomerata	II			
Stellaria media	II			
Veronica chamaedrys	II			
Cirsium helenioides	I			
Ranunculus bulbosus	I	Yes		
Carex nigra	I			
Juncus effusus	I	Yes	Yes	Yes
Rumex obtusifolius	I			
Geum rivale	I			
Deschampsia cespitosa	I	Yes		

Deschampsia cespitosa	I	Yes		
Carex panicea	I			
Caltha palustris	I			
Heracleum sphondylium	I			
Euphrasia nemorosa	I			
Centaurea nigra	I			
Cardamine pratensis	I			
Poa annua	I			
Festuca pratensis	I	Yes		
Agrostis capillaris	I	Yes		
Briza media	I			
Poa pratense		Yes		Yes
Juncus bufonius		Yes	Yes	Yes
Cirsium arvensis		Yes		
Helictotrichon pubescens		Yes		
Polygonum bistorta		Yes		
Chamerion angustifolium		Yes		
Urtica dioica			Yes	
Cardamine pratensis				Yes

Table 3.16 shows that as at Piper Hole a number of species including the wind dispersed *C. angustifolium* are found within the hay, manure and dung samples at New House farm which were not recorded amongst that meadow vegetation. In this case the number and species make up of the species within the manure and dung samples is somewhat similar. This suggests that less of the species at New House farm were falling directly into the manure from hay racks.

3.3.1 Comparison of New House and Piper Hole Results

The PCA plots (Figure 3.10a and 3.10b) show that the New House and Piper Hole vegetation differ in species composition. The Piper Hole meadows contain more *G. sylvaticum* and *S. officinalis* whereas the New House meadows contain more *R. minor*, *Festuca rubra* and *C. helenoides*. The manure samples from these two differing meadow systems contain far less variation and are in fact very similar, being dominated by *P. trivialis*. The hay samples of the two meadow systems differ far less than the vegetation as well and are generally intermediate in composition between the vegetation and the manure although still dominated by *P. trivialis*.

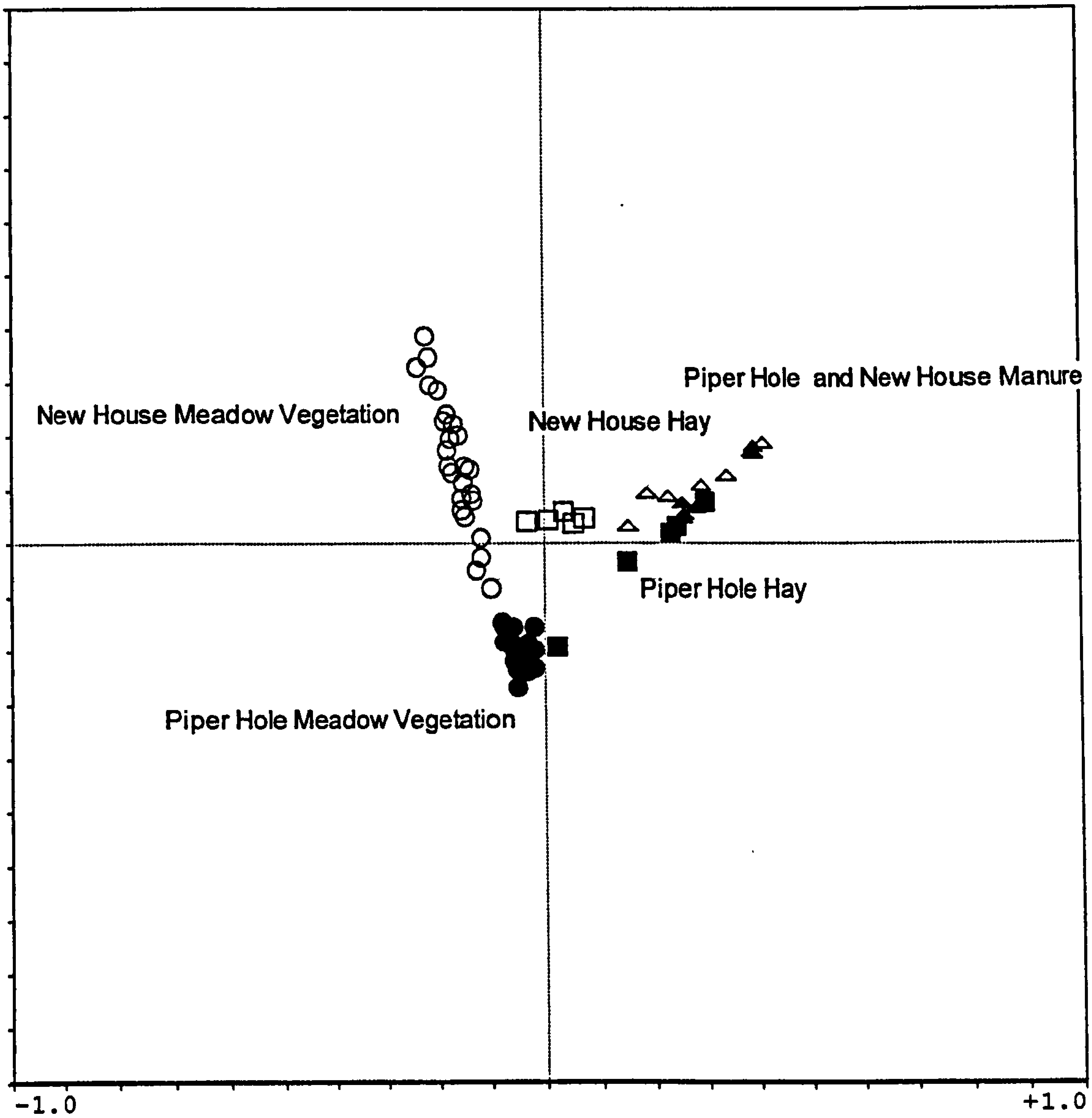


Figure 3.10a The PCA plot showing the site ordination for ○ New House Vegetation, ●Piper Hole Vegetation, □ New House Hay, ■ Piper Hole Hay, △ New House Manure and ▲Piper Hole Manure.

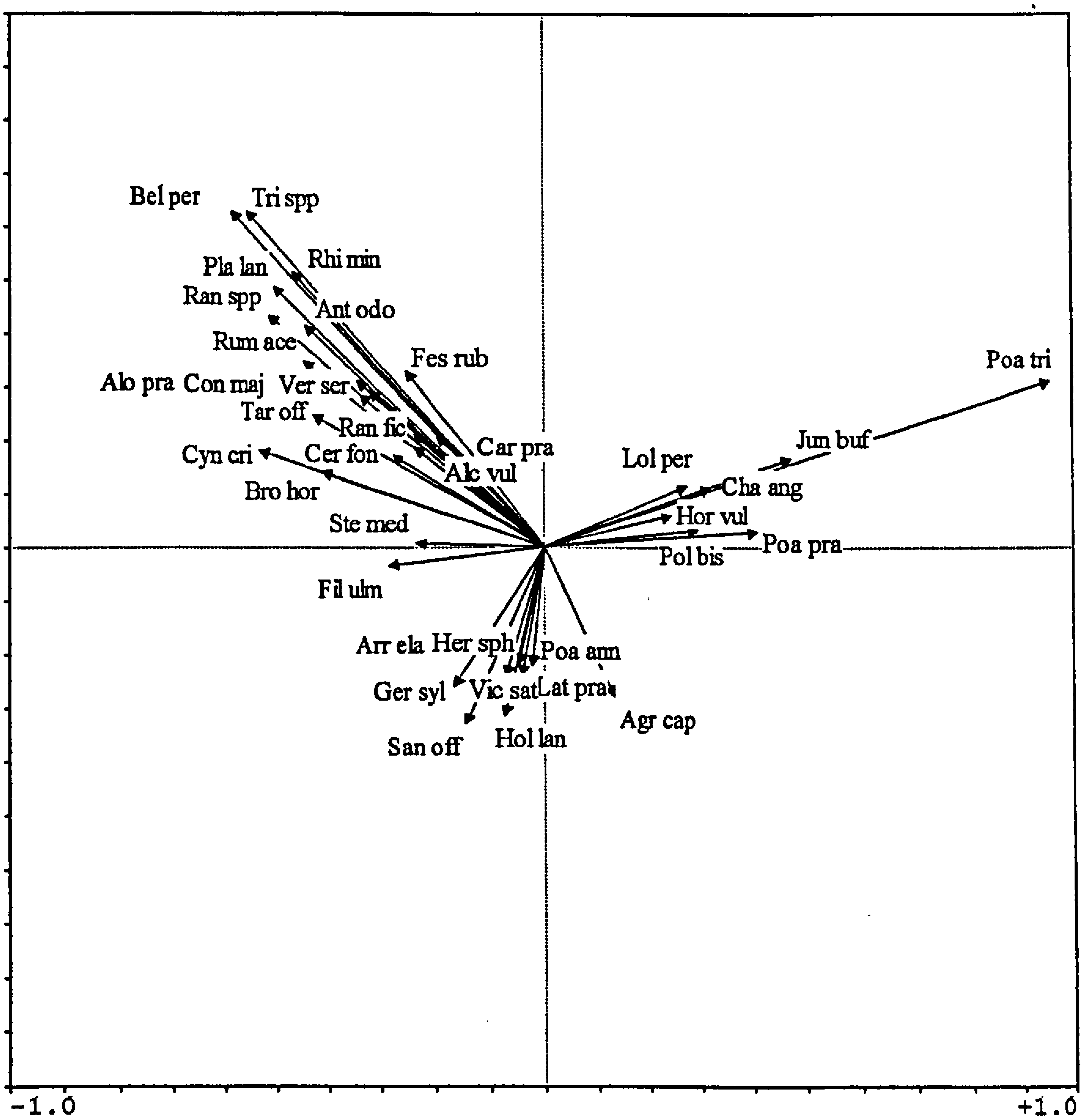


Figure 3.10b The PCA plot showing the species ordination for New House meadows vegetation, hay, manure and dung and Piper Hole meadow vegetation, hay, manure and dung.

3.4 Discussion

The results presented above show that despite hay being cut from diverse swards only a restricted range of species are incorporated into the hay that is fed to the stock over the winter. There are a number of reasons for this; early flowering species such as *Rhinanthus minor* and *Alchemilla glabra* for example will have either flowered and set seed prior to the hay being cut or shed all their seed during hay making, whilst others such as *Sanguisorba officinalis, Cirsium heterophyllum* and *Geranium sylvaticum* for example will have failed to produce ripe seed before the hay was cut. The range of species incorporated into the hay will therefore be a snapshot of those viable at the time of cutting. Some species may also have an increased propensity to remain on the stalk following cutting and turning.

The hay samples from both farms are dominated by seed of the grass *Poa trivialis.* In the phenology of northern hay meadows this species flowers mid season; from June to July (Hodgson *et al.,* 1995). Whilst not being a particularly dominant part of the vegetation in terms of percentage cover it is a fairly fecund species. Hodgson *et al.,* (1995) also note that *P. trivialis* does not have any specialised dispersal features but is widely dispersed by agricultural practices.

The number of species within the hay samples at New House farm was much lower than found at Piper Hole. *P. trivialis* the most dominant species in the hay from Piper Hole and the second most common species in the hay from New House Farm was found with the same frequency and abundance range in the vegetation whilst there was 845 seeds kg^{-1} in Piper Hole hay samples compared to only 90.9 seeds kg^{-1} in New House hay. The fact that early flowering species such as *Bellis perennis* and *P. lanceolata* were also found in similar quantities to *P. trivialis* at New House Farm could point towards the hay being cut at an earlier stage of sward maturity at this site. The hay cut dates would not show the extent of this as various factors could have an impact on the maturity of the vegetation such as New House Farm (~350 m) is at a higher altitude than Piper Hole (~260 m) and the hay samples were obtained in a different year with different weather conditions. The date at which the meadows were shut up and the grazing pressure prior to shut up are also key factors which could alter the maturity of vegetation at any given date. It is also possible that the hay making process could be different at the two sites for example the hay may have been turned more often at New House Farm although discussion with the farmers has failed to show any differences.

Wells, Frost and Bell (1986), studying hay from a species rich lowland meadow in Cricklade, Wiltshire, found a total of 41 species in 8 bales of hay. The mean number of species in each bale was 26 and in total an average of 450 000 seeds per 21 kg bale were reported. Whilst the mean number of species in each bale reported here is similar, a mean of 23 species per bale from Piper Hole and 16 at New House Farm, this represents a much greater number of seeds than was found in this chapter. At Piper Hole a 21 kg bale would be estimated to contain 33 915 seeds and only 7 875 seeds per 21kg at New House Farm. However, no germination or any method of checking viability was used in the methods of Wells, Frost and Bell (1986) and as such these results could be considered an over estimation with the germination of seeds within samples, as used here, an underestimation. The timing of hay cutting operations will be critical in determining the seed content of hay. Another factor which may have a considerable influence on the species present in hay bales is seasonal variation in seed production. Such behaviour was mentioned by Williams (1978) in the Park Grass experiment at Rothamsted and in chalk grasslands (Dickie 1977 in Wells, Frost and Bell, 1986) showed yearly variation in the region of 100 fold over 3 years.

Smith, Shiel and Pullan (1996) in a study of seed shed during the making of hay from a traditionally managed meadow in Upper Teesdale found a similar number of seeds kg^{-1} as Wells, Frost and Bell (1986). In this experiment again there was no germination or checking of viability of the seeds counted. The authors suggested that whilst Wells, Frost and Bell (1986) found mainly grasses within the hay the species which drop from the cut sward during hay making are mainly dicotyledonous herb species. However the majority of the dicotyledonous species were accounted for by species of little conservation value aside from the early flowering annual *Rhinanthus minor*.

Storage of the manure reduced the seed quantity and species composition of the manure. The reduction in seed quantity and species composition did not occur until after 3-6 months storage. It should be noted however that the manure used in this experiment was only stored in small tubs rather than in a large heap as would be the case at the farms from which it originates. It is likely that the rotting process may be more severe in larger heaps and when the seeds occur at different depths and this should be investigated further. Fresh farmyard manure from Piper Hole applied to meadows at 12 tonnes ha^{-1} would supply 2 220 000 seeds ha^{-1} falling to 288 000 seeds from year old manure whilst at samples from New House Farm suggest 259 440 seeds ha^{-1} when fresh and no seeds when 1 year old. In the years in which these samples were taken, the extent to which farmyard manure could be a vector for the dispersal of hay meadow plants and therefore play a role in the maintenance of the high botanical diversity of these meadows would appear to be limited to a relatively small number of species. However a number of species such as *P. trivialis* could be dispersed regularly by this method. Also a later hay cut may enable those species such as *G. sylvaticum* to be dispersed via farmyard manure. The perennial nature of these species means that the late hay cuts that occur in wetter years (Rodwell and Dring 2001) could be crucial to the dispersal of seed and subsequent maintenance of botanical diversity. Further experimentation using known quantities of certain species may help to understand to what extent this may be a possible means of dispersal. Certainly the cutting of grasslands at optimum points in order to create green "hay" for spreading onto meadows has been shown to be a helpful tool in the recreation of degraded grasslands (Mortimer *et al.,* 2002).

The seed content of fresh farmyard manure from these farms contained a similar range of species to that of the hay samples whilst the dung samples contained a far more restricted range of species. This suggests that the majority of seed found in fresh farmyard manure has fallen directly from the hayracks and become incorporated into the manure without passing through the digestive system of the cattle. Further investigation is needed however in order to establish whether species which did not occur in the hay samples due to the date at which it was cut could theoretically be dispersed via this route.

Two species of rush were found in the hay and manure samples as well as the dung samples. These species were found in the hay in small quantities and became more prominent (whilst not occurring in any greater quantity) in the older manure and the dung samples. It is possible that these species which occur in the wetter areas of the meadows are more resistant to the digestive and storage processes than other species. It is well known that rush species are capable of surviving for long periods in soil seed banks (Hodgson *et al.,* 1995) and it has also been suggested that those species with persistent soil seed banks are more likely to survive digestion by animals (Pakeman *et al.,* 2002). In the context of northern hay meadows it would be desirable to establish how the species that survive digestion and storage compare to those which are found within the soil seed bank.

Once farmyard manure is spread it is also necessary to gain and understanding of whether any seed contained within it either germinates or becomes incorporated into the soil seed bank. Also the effect of manure producing gaps within the vegetation and how these gaps are subsequently filled needs to be understood.

The results presented in this chapter have demonstrated that from hay with a restricted range of species of seed compared to the vegetation from which it originated a number of species such as *P. trivialis, Juncus effuses* and *J. bufonius* are capable of surviving digestion by cattle. Farmyard manure contains a similar range of species as hay suggesting that most of the seed found within manure falls directly onto the barn floor without passage through the digestive system of the stock and that storage of manure for up to 6 months has little effect on the viability of seeds but for periods longer than this large reductions in viability of seeds occur. In conclusion it is possible that manure could act as a medium for the dispersal of hay meadow seeds however the limited range of species found within the hay restricts the range of species for which this means of dispersal is possible.

4. The effects of *In Vitro* rumen digestion on seed viability.

4.1 Introduction.

The previous chapter demonstrated that hay samples, cut from diverse swards, contains seed from a range of species some of which were capable of surviving the digestive processes of cattle. However the broad survey approach adopted did not readily lend itself to establishing how individual species were affect by ruminant digestion. A number of species not found as seed within those hay samples may be capable of dispersal via this method given appropriate hay cut dates or hay making conditions. This chapter describes an *in vitro* laboratory method which mimics the digestion of cattle which was used to establish how seeds of certain species were affected by the digestive process.

Differences in seed morphology may result in differential seed survival rates following the ruminant digestive process so it is possible that some of the species found in hay meadows may be favoured by endozoochorous dispersal to a greater extent than others. Welch (1985) demonstrated that 88 species found in Scottish upland pastures were dispersed by ruminant animals (sheep and cattle) but only a small number of species were frequently dispersed. Variation in the effects of ruminant digestion could therefore have an effect on botanical diversity in hay meadows which could be either positive or negative.

Simao Neto *et al.*, (1987) concluded that it was the rate of passage that was critical to the recovery of viable seed but no single physical seed characteristic could be correlated with the rate of passage through the ruminant digestive system. It is therefore necessary to look at a range of plants common to hay meadows to see how germination is affected by the digestive process in order to asses whether this is likely to be a means of dispersal in the case of hay meadows.

The feeding of seeds to cattle, followed by subsequent recovery, has been used to determine the effects of digestion on seeds. Whilst providing an accurate assessment of the effects of digestion this "feed it and see" method is very slow and time consuming. It is also very difficult to accurately quantify how much seed is eaten and to control the individual species of seed that is ingested. In order to asses the effects of rumen digestion on controlled quantities of individual plant species seed it is necessary to use either an *in vitro* method or an *in sacco* method.

In sacco methods as used by Simao Neto and Jones (1987) for example involve placing samples (in this case seeds) in a nylon mesh bag and then inserting the sample directly into a rumen fistulated animal. This method provides an accurate representation of rumen digestion. If rumenal digestion is the part of the system mainly responsible for changes to seed viability this would be a suitable method for assessment of seed resistance to digestion. However, whilst providing an accurate and easily controllable method, welfare issues aside, this method does not allow pre and post rumen digestion to be assessed

The experiment reported here made use of an *in vitro* digestion technique, first developed by Tilley and Terry (1963). This technique is a less time consuming and cheaper method for the assessment of the ruminant digestion on forages than either of those discussed above. Fredrickson *et al.,* (1997) showed similar results using both *in vitro* and *in sacco* methods to asses the effects on seed viability with the grass *Erogrostis lehmanniana* demonstrating its reliability at representing rumen digestion. It also has the advantage of allowing pre- and post-rumen digestion to be included in the experimental procedure.

The aim of this experiment was to assess the potential of endozoochory as a dispersal mechanism involved in maintaining the botanical diversity in traditionally managed meadows of the Pennines. In particular it is proposed that varying proportions of seed from differing hay meadow plants will remain viable following digestion by cattle.

4.2 Methods.

The laboratory methods used in this study are an adaptation of those described by Tilley and Terry (1963); this method is regularly used to estimate the *in vitro* dry matter disappearance of forages. In this case, however, the only response variable of interest was the subsequent viability of the recovered seeds. 1g samples of each of the species chosen for study were subjected to a 3 stage digestive process.

The first stage involved abrasion of the seeds with sand paper. This is an extension of the Tilley and Terry (1963) method which is carried out on ground forages. This process was designed to mimic the mastication effects of cattle and took place for 0, 30 or 60 seconds. Following seed abrasion, the seeds were incubated at 39°C under anaerobic conditions in freshly collected rumen fluid. The rumen fluid incubation phase which was designed to represent rumen fermentation, took place for 0, 12, 24, 48 and 72 hours. Finally the seed samples were divided in half and incubated at 39°C in acidified pepsin solution for either 0 or 6 hours. Acidified pepsin solution incubation was included in order to incorporate a post ruminal enzymic digestive stage into the experiment.

The entire experiment was designed in a factorial manner so that the effects of all the combinations of the abrasion and incubation times could be observed. This gave a total of 30 samples for each species tested. There were two replicates.

4.2.1 Choice of Species.

Seeds of the grasses *Poa trivialis* and *Anthoxanthum odoratum* and the dicotyledonous herbs *Sanguisorba officinalis, Myosotis arvensis* and *Filipendula ulmaria* were obtained from British Seed Houses PLC, Portview Road, Avonmouth, Bristol, BS11 9JH. These species were chosen as being representative of a range of plants found in Pennine Hay Meadows. Previous study had determined that *Poa trivialis, Anthoxanthum odoratum* and *Myosotis sp.* were found in reasonable quantities in manure from traditionally managed meadows whereas the two perennial herbs *Sanguisorba officinalis* and *Filipendula ulmaria* were not represented. However a late hay cut date may enable such species to have seeds become incorporated into the hay. Investigation of the effects digestion on such species is therefore necessary.

4.2.2 Abrasion of Seeds.

To simulate the effect of chewing, 1g samples of each species were rubbed gently between two pieces of coarse sandpaper (3M General Purpose Sand Paper, Course) for 0 seconds, 30 seconds or 60 seconds.

4.2.3 Collection of Rumen Fluid.

The rumen fluid was collected from a freshly slaughtered cow at a local abattoir and transported back to the lab in a pre-warmed flask. On returning to the lab it was filtered through 4 layers of muslin cloth into a bottle. In order to maintain the microbiological properties the contents were then flushed from above the surface of the fluid with CO_2 in order to maintain anaerobic conditions and the rumen fluid mixed with a pH buffer solution and stored at 39°C until needed.

4.2.4 Preparation of Rumen Buffer Solution.

All reagents listed in Table 4.1 except $CaCl_2$ were dissolved through constant stirring using a hot plate set at 38-39°C. $CaCl_2$ was added last after the other reagents had dissolved. When the reagents were dissolved the solution was made up to 1 litre with distilled water. The solution was then flushed from above with CO_2 until it became clear.

Table 4.1. Chemical composition of 1 litre of buffer solution.

Reagent	Weight (g)
$NaHCO_3$	9.8
$Na_2HPO_4.12H_2O$	9.3
NaCl	0.47
KCl	0.57
$MgCl_2$ anhydrous	0.06
$CaCl_2$ anhydrous	0.04

4.2.5 Incubation In Rumen Fluid.

Following the abrasion treatment the seeds were transferred to boiling tubes to which 50 ml of the buffered rumen fluid (10 ml of rumen fluid and 40 ml of buffer solution) was added. The tubes were then flushed with CO_2 and a Bunsen gas release valve inserted into the top of each of these tubes. Following a gentle shake the tubes were placed in a water bath set at 38-39°C.

The tubes were then incubated for 0, 12, 24, 48 or 72 hours. The rumen fluid reaction was stopped at these times using 1ml of 5% $HgCl_2$ solution. In order to separate the seeds, the samples were then immediately washed, filtered over a vacuum and divided into half by weight.

4.2.6 Incubation in acidified pepsin solution.

Half of each of the seed samples were then incubated at 39°C in a water bath for 6 hours in an acidified pepsin solution (3mL of 2.42M HCl mixed 1 ml of 50 g L^{-1} pepsin whilst the other half was left untreated.

4.2.7 Germination of seeds.

After the final treatments had been imposed the seeds were immediately filtered over a vacuum, washed with distilled water and placed on moistened filter paper (Whatman Number 1) in petri dishes for one month. The Petri dishes were spread out in a growth room set at 20^0C during an 18 hour day and 15^0C during the 6 hour night. The germinating seeds were counted and removed on a daily basis for the first week and then weekly for a further 4 weeks.

4.2.8 Statistical Analysis.

Results for each species were examined separately. With the exception of *M. arvensis* the data were analysed using Generalised Linear Models (GLM) in Minitab for Windows 13.3. The model included the factors abrasion time, rumen incubation time, acidified pepsin incubation and the interactions between them. Tukey Tests at 95% confidence levels were used to determine the individual differences between treatments. Residuals were examined for normality using the Andersen Darling Test and where necessary data were transformed, by use of a square root function.

The analysis of the data for *M. arvensis* was carried out differently. This was necessary because of the very low germination rates following pepsin incubation which gave a high proportion of zeros in the data set causing it to be skewed. Consequently, data for seeds treated or not treated with pepsin were combined together. A square root transformation was then performed prior to analysis for the factors abrasion and rumen fluid incubation using GLM as above.

4.3 Results.

All the species studied showed large reductions in the germination of the seeds following the complete *in vitro* digestive procedure. However there are differences in the responses of species to each of the 3 stages of the procedure. The results for each species are presented individually in the following sections.

4.3.1 Sanguisorba officinalis.

Germination of *Sanguisorba officinalis* seeds, one of the two perennial herbs tested in this experiment was greatly reduced by the complete digestive process. The completely untreated control samples gave rise to a mean value of 66.5 (±8.50) seeds germinated compared to a mean of 10.5 (±0.50) seeds germinating following the longest abrasion and rumen fluid incubation treatment followed by pepsin incubation; i.e. 60 seconds abrasion, 72 hours rumen incubation and 6 hours pepsin incubation. Abrasion time had no significant effect on germination of *S. officinalis* seeds ($F_{2,59}$=0.12, P=0.888).

Rumen incubation caused a significant ($F_{4,59}$=26.20; P=<0.001) decline in the germination rate of *S. officinalis* seeds. Figure 4.1 shows that the longer the period of rumen incubation the greater was the decline in seed germination. A relatively short period (12 hours) more than halved the number of viable seeds. Another major reduction in germination occurred at 48 hours and beyond.

Acidified pepsin incubation had a significantly negative effect on *S. officinalis* seed germination ($F_{1,30}$=85.22; P=<0.001) which is shown in Figure 4.1.

The interactions between the treatments on seed germination were examined and only rumen incubation and pepsin incubation showed a significant effect ($F_{4, 59}$=5.23; P=<0.001). This interaction is shown in Figure 4.1. For seeds which did not subsequently undergo pepsin incubation, there was a substantial decline in germination capacity following progressively longer periods of rumen incubation.

However, pepsin incubation reduced the germination of seeds to such an extent that the previous rumen incubation time was largely irrelevant.

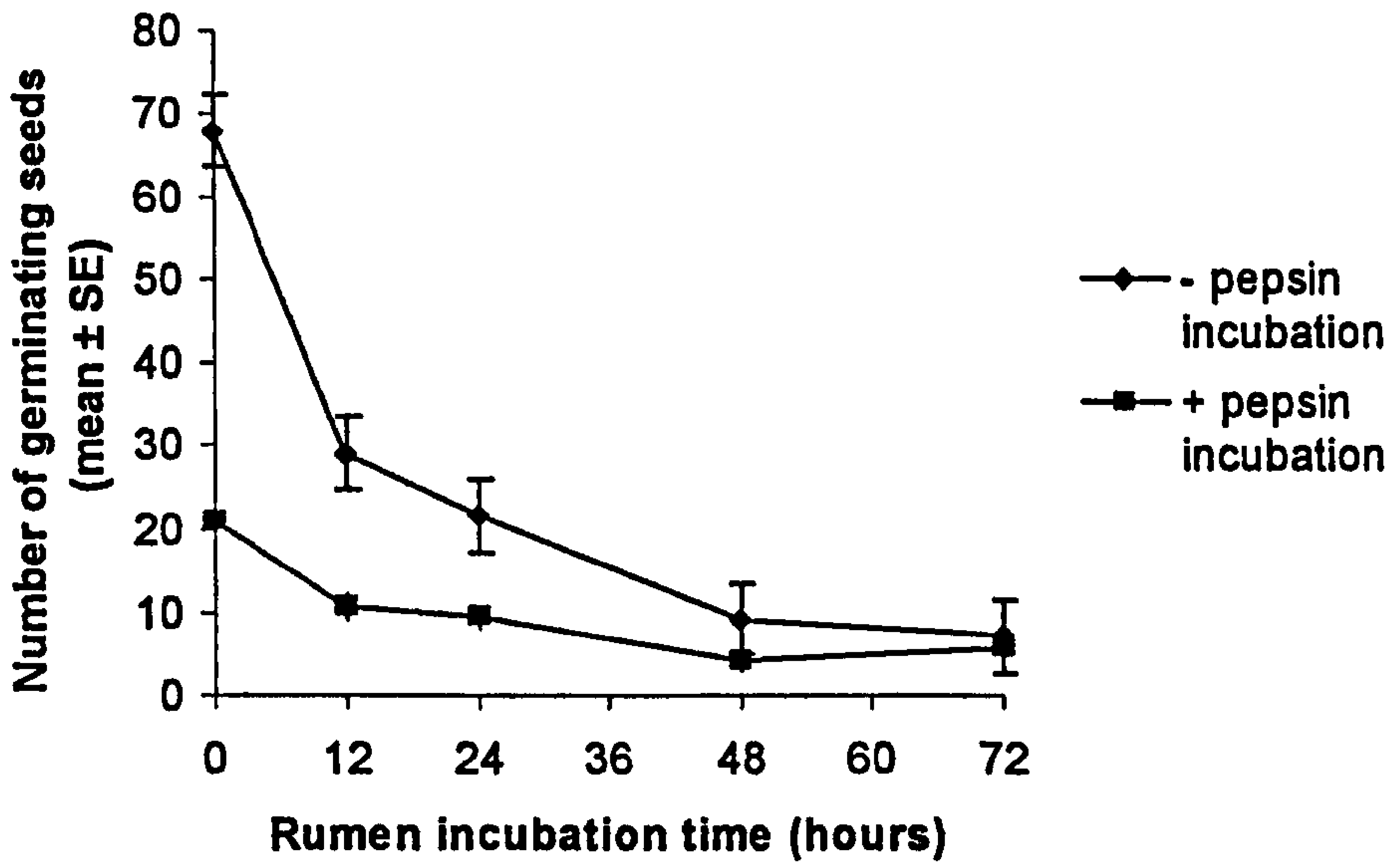


Figure 4.1. The interaction between rumen fluid incubation and pepsin incubation for *Sanguisorba officinalis* seed germination.

The overall effects of the digestive processes examined in this experiment suggest that whilst *S. officinalis* seeds may well survive mastication and passage through the rumen; post ruminal enzymic digestion is likely to greatly reduce the probability of seeds remaining viable following digestion. Nevertheless, a very small number of seeds did remain viable.

4.3.2 Filipendula ulmaria.

Germination of seeds from the perennial herb *F. ulmaria* was much reduced by the digestive process. The completely untreated control samples gave a mean value of 24.5 (±7.50) seeds germinated compared to a mean of 2.5 (±2.5) seeds germinating following 60 seconds abrasion, 72 hours rumen incubation and 6 hours pepsin incubation. Comparisons of the means for the different abrasion times showed no significant differences ($F_{2,59}$=1.48; P=0.238).

As was seen with *S. officinalis*, incubation of *F. ulmaria* seed in rumen fluid gave rise to a highly significant reduction in germination ($F_{4,59}$=8.01; P=<0.001). The reduction in germination occurred only after 48 hours rumen incubation; however 72 hours incubation had no further significant effect. Up until that point rumen fluid has no discernable effect on the number of seeds germinating. This effect is seen in Figure 4.2. Incubation in acidified pepsin solution significantly reduced seed germination ($F_{1,59}$=74.27; P=<0.001). This effect is shown in Figure 4.2.

As was seen with the other perennial herb species, the only treatment interaction that showed a significant effect was rumen incubation and pepsin incubation ($F_{4,59}$=6.85; <P=0.001). The interaction of these treatments is shown in Figure 4.2. For seeds not treated with acidified pepsin incubation a reduction in germination was seen following only following 48 and 72 hours rumen incubation. For seeds undergoing the acidified pepsin incubation a large reduction in germination was seen irrespective of the previous rumen incubation treatment.

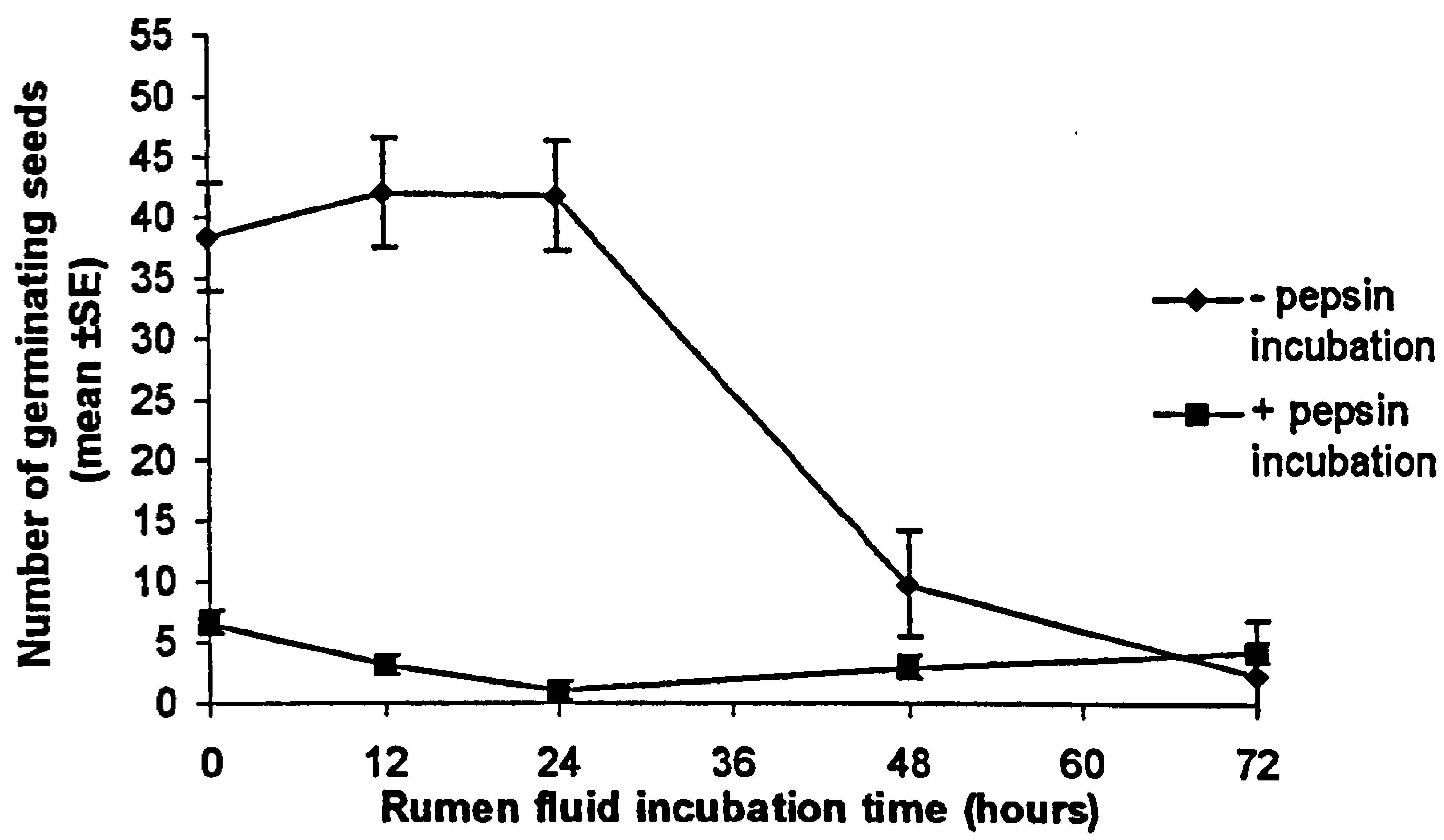


Figure 4.2. The interaction between rumen fluid incubation and pepsin incubation for *Filipendula ulmaria* seed germination.

Both *S. officinalis* and *F. ulmaria* the two perennial herbs tested in this experiment showed similar responses. In both cases abrasion of the seeds did not significantly reduce the germination of the seeds. Both *S. officinalis* and *F. ulmaria* seeds show a significant negative response to rumen incubation and pepsin incubation.

The interaction between rumen fluid incubation and pepsin incubation is also significant with both of these species. In both cases pepsin incubation alone reduced the germination of the seeds to a low level and this was not affected by prior rumen incubation. However *S. officinalis* showed an almost linear decline in germination rate as rumen fluid incubation time increased whereas *F. ulmaria* germination remained almost constant up until 24 hours incubation, germination only fell after 48 and 72 hours incubation.

4.3.3 Anthoxanthum odoratum.

The complete digestive process greatly reduced the subsequent germination of *A. odoratum* seeds. A decline from a control mean of 89.5 (±79.5) to a mean of 5.0 (±5.0) following 60 seconds abrasion, 72 hours rumen incubation and 6 hours pepsin incubation was recorded. The results for *A. odoratum* in general were less clear and less consistent than the other species tested.

Abrasion had a significant effect on the germination of *A. odoratum* seeds ($F_{2,59}$=6.49; P=0.003). A decline in germination rates was recorded after 30 seconds abrasion but no further decline was bought on by further abrasion, as is shown in Table 4.2.

Table 4.2 Mean ± SE (n=20) number of *Anthoxanthum odoratum* seeds germinating following 3 abrasion times.

Abrasion Time (seconds)	Mean (±0.69)
0	54.7[a]
30	30.70[b]
60	28.20[b]

Rumen fluid incubation was found to have a significantly negative effect on the germination of *A. odoratum* seeds ($F_{4,59}$=3.55; P=0.02). Figure 4.3 shows that this reduction in germination only occurred after 72 hours incubation. *A. odoratum* was also significantly ($F_{1,59}$=4.43; P=0.040) reduced in germination following acidified pepsin incubation.

No significant treatment interactions were found with the analysis of the *A. odoratum* data.

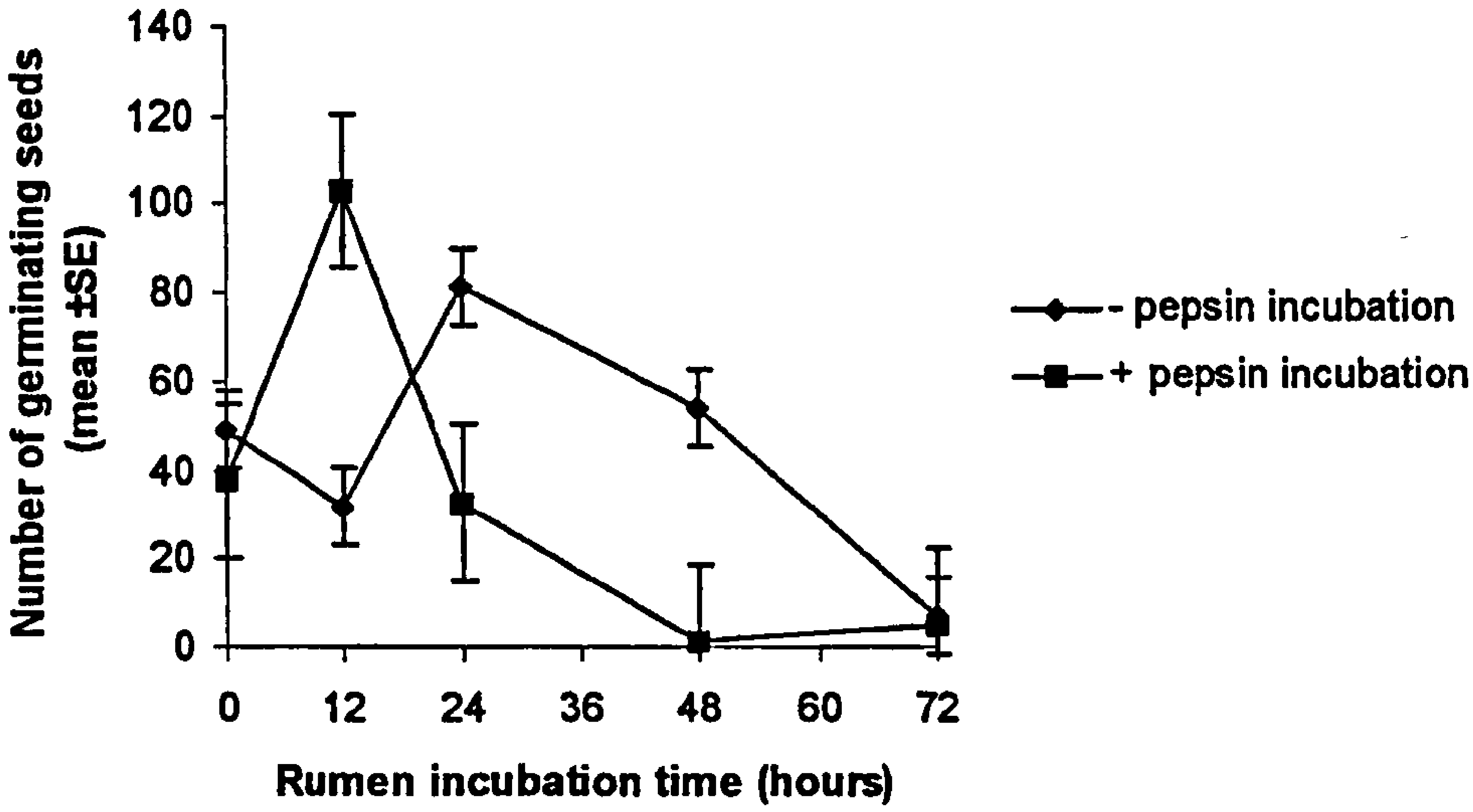


Figure 4.3. The interaction between rumen fluid incubation and pepsin incubation for *Anthoxanthum odoratum* seed germination.

4.3.4 Poa trivialis.

P. trivialis showed a reduction in germination from a mean of 735.5 (±178) for the control samples to 0.0 for the samples which were subjected to 60 seconds abrasion, 72 hours rumen incubation and 6 hours pepsin incubation.

A significant response to abrasion ($F_{2,59}$=10.85; P= <0.001) was recorded for *P. trivialis*. The response, shown in Table 4.3, is negative. Only 60 seconds of abrasion gave rise to a significant reduction in germination.

Table 4.3 Mean ± SE (n=20) number of *Poa trivialis* seeds germinating following 3 abrasion times.

Abrasion Time (seconds)	Mean (±3.50)
0	245.6[a]
30	127.9[a]
60	58.0[b]

When compared to the means of all the other treatments, rumen fluid incubation showed a significant ($F_{4,59}$=16.53; P= <0.001) negative effect on seed germination. As can be seen from Figure 4.4 a reduction in germination was only recorded following 48 hours rumen incubation and further incubation did not increase this reduction. Acidified pepsin incubation when compared to the means of all the other treatments showed a significant negative effect on seed germination ($F_{1,59}$=14.11; P=<0.001).

The interaction between rumen incubation and pepsin incubation for *P. trivialis* was significant ($F_{4,59}$=3.63; P=0.013). Acidified pepsin incubation reduced germination to such an extent that prior rumen fluid incubation was largely irrelevant.

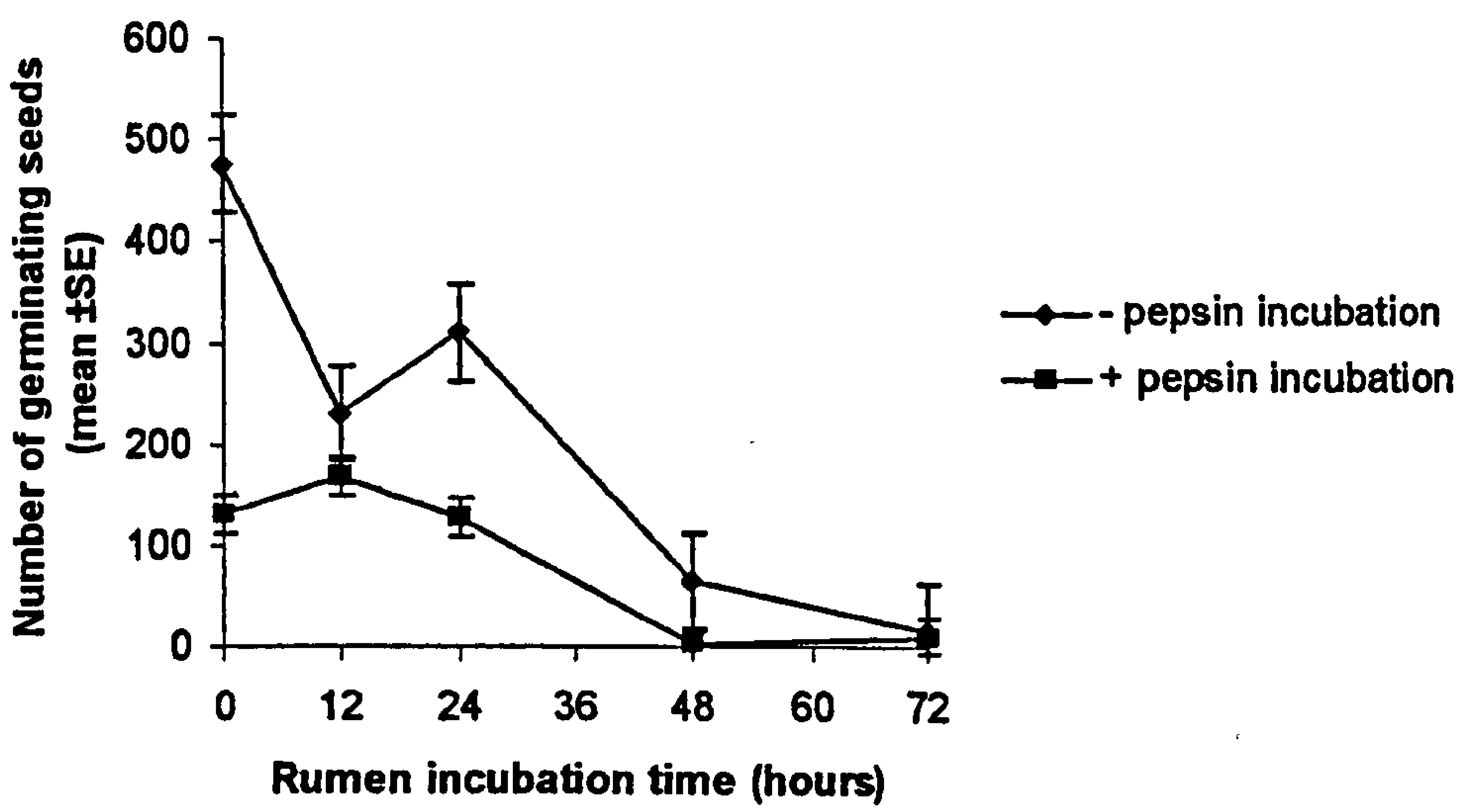


Figure 4.4 The interaction between rumen fluid incubation and pepsin incubation on *P. trivialis* seed germination.

4.3.5 Myosotis arvensis

The overall effect of the digestive process was to reduce the levels of germination from 46.5 (±23.5) to 2.5 (±2.5) in the case of *M. arvensis*. The effect of abrasion was not statistically significant ($F_{2,29}$=2.62; P=0.095).

Incubation in rumen fluid caused a significant ($F_{4,29}$=6.63; P= 0.001) reduction in the germination rate of *M. arvensis* seeds. This reduction, shown in Figure 4.5, occurred after only 12 hours incubation. Following 48 hours incubation germination was reduced almost completely.

Due to the very marked reduction in germination of *M. arvensis* it was not possible to carry out analysis of variance on the effects of pepsin or its interaction with the other variables. However it can be seen from Figure 4.5 that pepsin incubation caused an almost complete germination failure, which was not affected by previous rumen incubation.

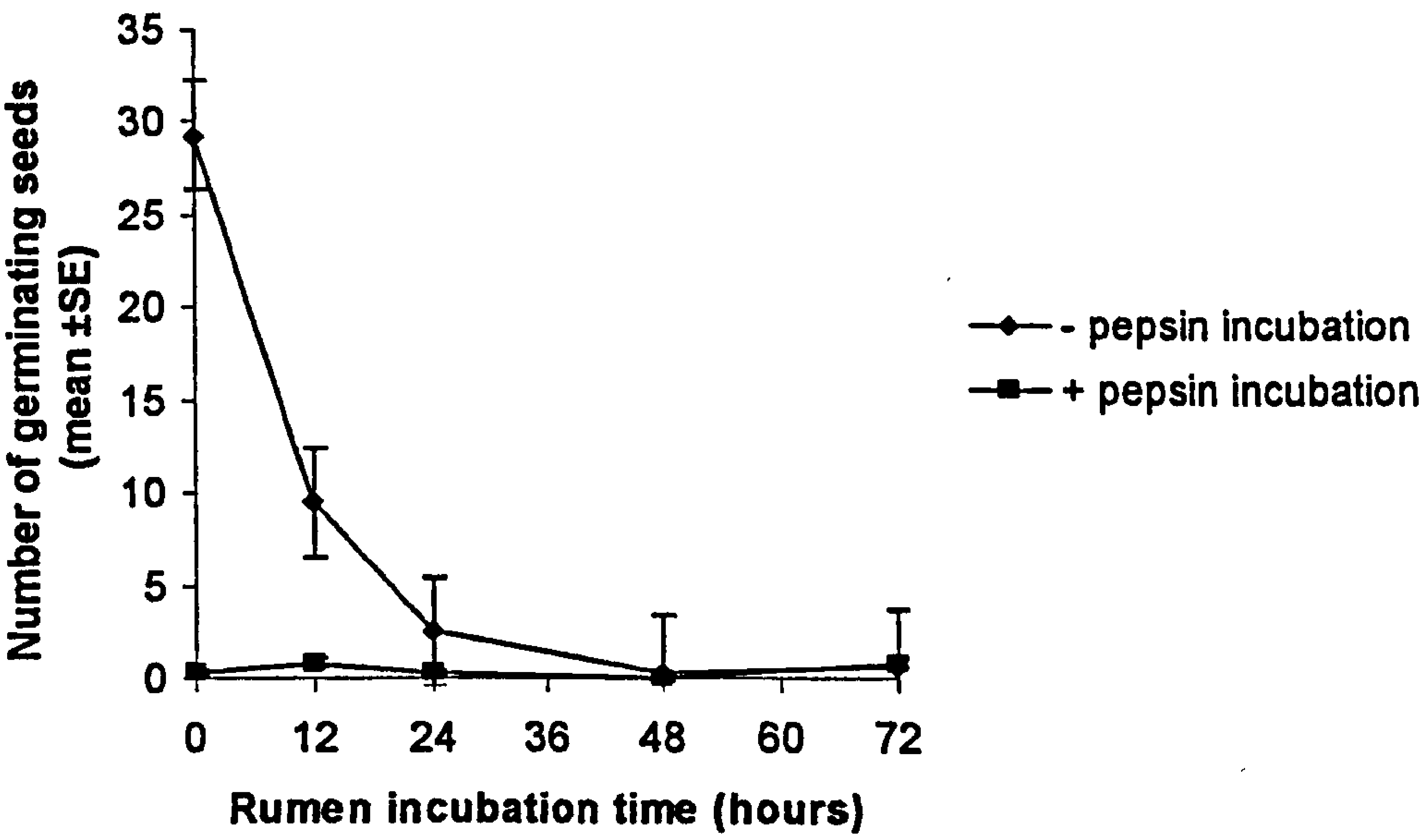


Figure 4.5. The interaction between rumen fluid incubation and pepsin incubation for *Myosotis arvensis* seed germination.

4.4 Discussion

All the species tested showed a reduction in germination following the entire simulated digestion process. Both rumen fluid incubation and pepsin incubation reduced germination. However, there were variations in the response of species to each component of the process. *Poa trivialis* and *Anthoxanthum odoratum*, both of the grass species, were the only species to show a statistically significant negative response to abrasion. Further work would need to be carried out to find out if this could be a more general pattern for grasses. Fredrickson *et al.*, (1997) using a fistulated sheep to recover masticated bolus, showed that only 35% of seeds of the grass *Eragostis lehmanniana* remained undamaged (seed coat remained intact) following mastication by sheep.

Rumen fluid incubation gave rise to a significant decline in germination for all the species tested. Within this overall effect however differences between species are apparent. *Sanguisorba officinalis, P. trivialis* and *Myosotis arvensis* all showed declines in germination rates following only 12 hours incubation whereas *F. ulmaria* and *A. odoratum* did not show a decline in germination rates until 48 and 72 hours respectively.

Kaske and Englehart (1990) state that seed size, density and shape are determinants of the time a seed is exposed to ruminal digestion and Ocumpaugh *et al.*, (1995) found that recovery of viable seeds increases when time in digestive tract is lower. It is possible therefore that whilst in this experiment *M. arvensis* seeds were the most damaged by the simulated digestive process, being the smallest seeds with no projections on the seed coat they would in actual fact pass through the digestive tract of cattle quicker and be more capable of surviving ingestion whilst *A. odoratum* having larger seeds with two distinct awns may take longer to clear the digestive system. In support of this theory Pakeman *et al.*, (2002) in a study looking at germination of seed from the dung of rabbits and sheep from various sites in the UK demonstrated that species that germinated were characterised by small seed size and rounder seeds.

Acidified pepsin incubation caused significant declines in germination rates for all the species tested whilst, *M. arvensis* showed an almost total failure to germinate following pepsin incubation. This strong effect of pepsin incubation on seed viability is similar to that reported by Remesova (2000).

Blackshaw and Rode (1991) showed that survival of grass seed subjected to rumen digestion tended to be less than that of broad leaved species in 12 species of weed commonly occurring in Canadian prairies. The results presented here are not as clear cut. Whist the dicotyledonous herb *F. ulmaria* showed more resistance to the simulation than the grass *P. trivialis*, both *M. arvensis* and *S. officinalis* were not found to be any more resistant and the seeds of the grass *A. odoratum* were the most resistant.

Any consistent pattern between dicots and monocots could only be elucidated through further experimentation with a wider range of species. It should be noted however that the results obtained for *A. odoratum* were the most inconsistent. Whilst it is possible that this could be due to natural variation in the seed coat properties of this species it was observed that during incubation despite repeated shaking these seeds were able to float to the surface of the fluid. Repeating the experiment with a rotating incubation method may provide more consistent results. These results do however compare favourably with those of Welch (1985) who, using field experimentation, showed that in a Scottish upland setting *A. odoratum* was one of the grasses most frequently introduced by dung deposition. An increase in the number of establishments was associated with heavy dung deposits.

Whilst these results show that digestion by cattle is particularly damaging to the viability of these seeds, it is worth noting that a small percentage of all, with the exception of *M. arvensis*, the species tested remained viable. Therefore whilst it is possible that these species could still be dispersed via this method it would not be likely to be a significant factor in the maintenance of overall botanical diversity in hay meadows. However, further work still needs to be done on how storage of the manure, which could be for 6 months to a year, would affect seed viability. Also, farmyard manure will contain large quantities of seed that has not passed through the digestive tract of the animals but has fallen directly into the manure from hay racks. This route of dispersal also needs to be investigated.

5. The effects of storage within the manure heap on seed viability.

5.1 Introduction.

It has been shown in chapter 3 that it is possible for a large quantity of viable seed to become incorporated into farmyard manure. This can occur directly as a result of seeds dropping onto the barn floor from the hay fed to housed animals. It was also shown in chapter 4 that a small proportion of the seeds ingested by the animals will remain viable to become incorporated into the manure.

If seeds are to remain viable when spread within the manure, they must be able to withstand the rotting processes that occur within the manure from the time of incorporation into the manure to the time when the manure is spread. Once spread there is the opportunity for the seed to either germinate immediately or to form part of the soil seed bank. In the traditional systems, farmyard manure is stored outside in middens. These comprise large heaps which are added to over the winter, but otherwise remain largely undisturbed. The manure remains heaped from the time it is removed from the barns, which occurs daily from autumn, till the time it is required for spreading, usually in the spring. During this time any viable seed that is within the manure may be subject to high temperatures and other rotting processes (Rupende *et al.,* 1998).

The method of storage of manure samples described in chapter 3 did not readily allow the effects on individual species to be assessed. Also the manure was not stored in large heaps which may have reduced the effectiveness of the rotting process. The purpose of this experiment was therefore to determine if seeds of individual species are capable of surviving an extended period of storage such that they are still viable when spread onto the meadows with the manure in the spring. The experiment addresses the following questions:

1. Are seeds of chosen hay meadow plants capable of germination following storage within a manure heap for 3, 6 and 12 months?
2. Is the ability of seeds to germinate following storage within a manure heap affected by the depth at which they are stored?
3. Are seeds of different species affected by the storage process differently?

Previous studies had determined that the species *Poa trivialis, Anthoxanthum odoratum* and *Myosotis spp.* are found in reasonable quantities in manure from traditionally managed meadows whereas the two perennial herbs *Sanguisorba officinalis* and *Filipendula ulmaria* are not as well represented. The experiment aims to establish whether these differences can be attributed to differential survival of storage within farmyard manure heaps.

5.2 Methods.

5.2.1 Seed Selection.

Seeds of the grasses *P. trivialis* and *A. odoratum* and the dicotyledonous herbs *S. officinalis, M. arvensis* and *F. ulmaria* were obtained from British Seed Houses PLC, Portview Road, Avonmouth, Bristol, BS11 9JH. These species were chosen as being representative of a range of plants found in Pennine Hay Meadows.

5.2.2 Seed storage.

1. A manure heap of approximately 2 m^3 was constructed at Cockle Park Farm, Northumberland. Fresh manure from the cattle barn was used. The manure heap used timber on 3 sides to maintain the structure.
2. Forty 0.5 g seed samples were weighed out and secured within nylon mesh bags (200 μ mesh size).
3. For each of the 5 species of seed used five 0.5 g samples were tied together in bundles. Each bundle had a section of twine to be left trailing to the surface to enable easy relocation of the samples.
4. Half of these bundles were then placed within the manure heap at a depth of 1m with the other half buried to 0. 5m.
5. Following 3, 6 and 12 months a bundle of each species from each depth was removed and the seeds germinated.
6. Five control samples of 0.5 g were germinated without any manure storage treatment for each species.
7. Including control samples there were therefore five 0.5 g samples stored for 0, 3, 6, 12 months at two different depths giving a total of 40 samples for each of the five species.

5.2.3 Germination of seeds following storage.

After retrieval of the seed bundles from the manure heap the seeds were removed from the nylon bags and immediately washed with distilled water, filtered over a vacuum and placed on moistened filter paper in petri dishes for one month. The petri dishes were spread out in a growth room set at 20°C during an 18 hour day and 15°C during the night. The germinating seeds were counted and removed on a daily basis for the first week and then weekly for a further 4 weeks. Control data were collected from seeds which had received no pre-treatment.

5.2.4 Data Analysis.

The data were analysed separately for each species. Due to different seed survival between data sets it was not possible to analyse each data set in the same manner. For *P. trivialis* the data were analysed using Generalised Linear Models (GLM) in Minitab for Windows 13.3. The model included the factors time of storage, depth of storage and the interaction between the two. Tukey Tests at 95% confidence levels were used to determine the individual differences between treatments. Residuals were examined for normality using the Andersen Darling Test.

With *A. odoratum and S. officinalis* the large number of zeros from the data of seeds stored more deeply gave rise to a non normal distribution of the data set. The data set was examined using GLM for the effects of storage time alone, by omitting the depth treatment factor. Tukey Tests at 95% confidence levels were used to determine the individual differences between the lengths of storage. One way analysis of variance was then used to examine the data set for differences between the two depths of storage. Residuals were examined for normality using the Andersen Darling Test and where necessary the data were transformed using a square root function. A similar approach was used with the *F. ulmaria* data set except the very low germination rates following 12 months storage in the manure heap meant statistical analysis was not possible.

Due to the almost complete lack of germination of *M. arvensis* seeds following storage periods longer than 3 months it was only possible to analyse the square root transformed data from 3 months storage. The data from the longer storage times are simply presented in tabular and graphical form without any statistical information.

5.3 Results

5.3.1 *Poa trivialis.*

The longer *P. trivialis* seeds were stored, the less seeds germinated ($F_{3,39}$=118.26; P <0.001). As shown in Figure 5.1, 3 months, 6 months and 12 months storage gave rise to significantly lower rates of germination than no storage. Six and twelve months storage also gave significantly lower germination rates than 3 months storage, however there was no significant difference between six and twelve months storage.

Seeds of *P. trivialis* which were stored at a depth of 1 m from the surface of the manure heap were significantly ($F_{1,39}$=24.85; P<0.001) reduced in germination capacity compared to those stored at a depth of 0.5 m.

A significant, $F_{3,39}$=8.79; P<0.001, interaction was found between length of storage and depth of storage for *P. trivialis* seeds. Figure 5.1 shows that germination was significantly reduced with increased storage time but that the extent of the reduction was greater when the seeds were stored more deeply within the manure heap.

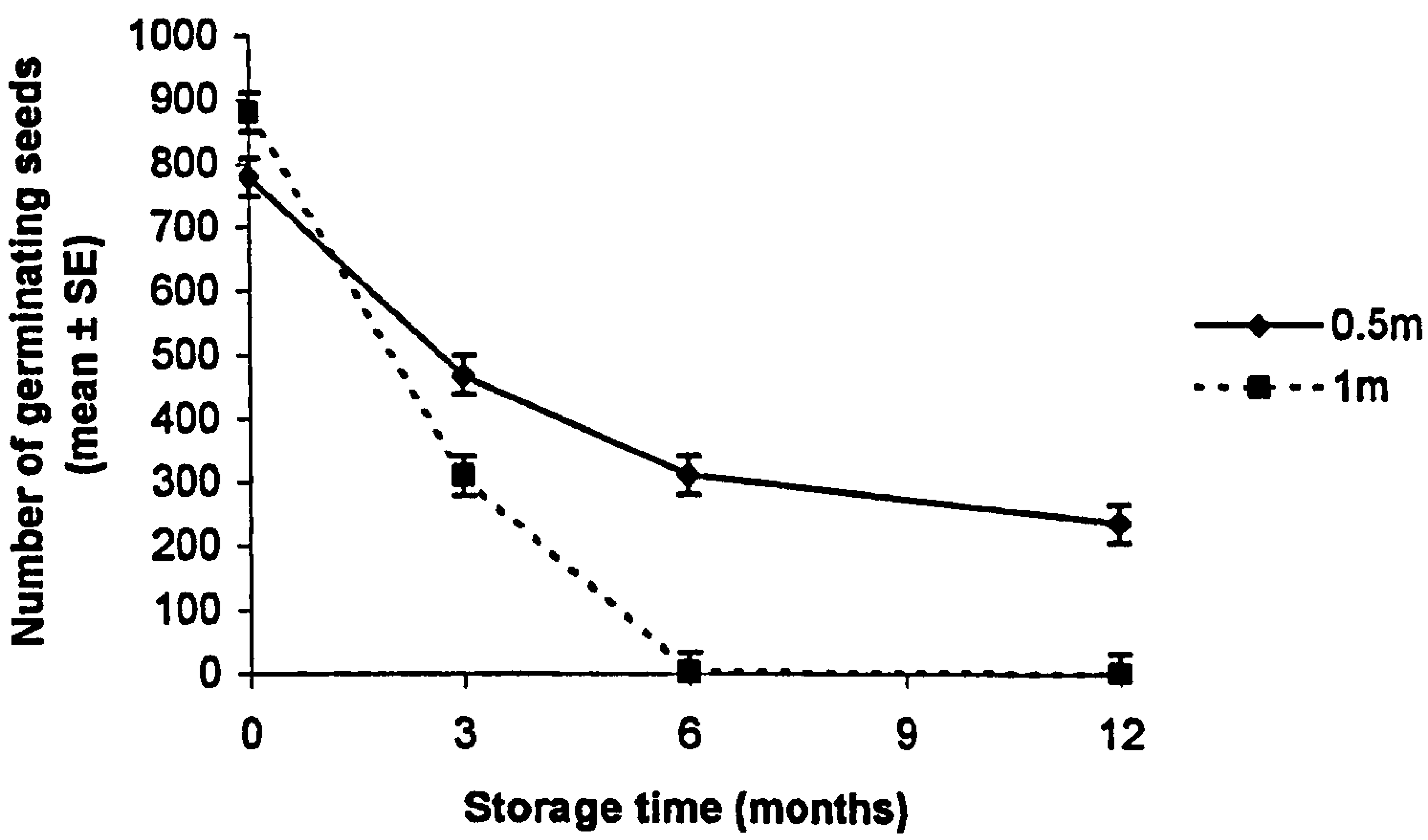


Figure 5.1 The influence of storage time on the germination of *Poa trivialis* seeds at two different storage depths.

5.3.2 Anthoxanthum odoratum.

Storage of *A .odoratum* seeds within the manure heap had a significant ($F_{3,39}$=8.87; P <0.001) negative effect on subsequent germination. As can be seen in Figure 5.2 this reduction was only seen after 12 months storage.

Seeds germinated after 3 and 6 months storage, but germination capacity was greatly reduced after 12 months of storage. The results obtained following storage at a depth of 1 m were not normally distributed; therefore it was not possible to use analysis of variance. This was due to the complete failure of germination following 6 and 12 months storage. At this storage depth there was however no increase in germination following storage for 3 months as was seen in the samples stored at 0.5 m.

As is shown in Figure5.2 the deeper seeds of *A. odoratum* were stored within the manure heap the lower the germination rates were. This effect was statistically significant, $F_{1,39}$=12.03; P<0.001.

The interaction of storage time with storage depth is shown in Figure 2. A storage time of 3 months had little effect on germination when the seeds were stored at a depth of 1 m. However when the seeds were stored at a depth of 0.5 m an increase in germination was recorded, this increase was also seen, although to a lesser extent, following 6 months storage. Seeds stored deeper within the manure heap after 6 and 12 months had virtually zero germination. Following 12 months storage the very low germination rate was seen at both storage depths.

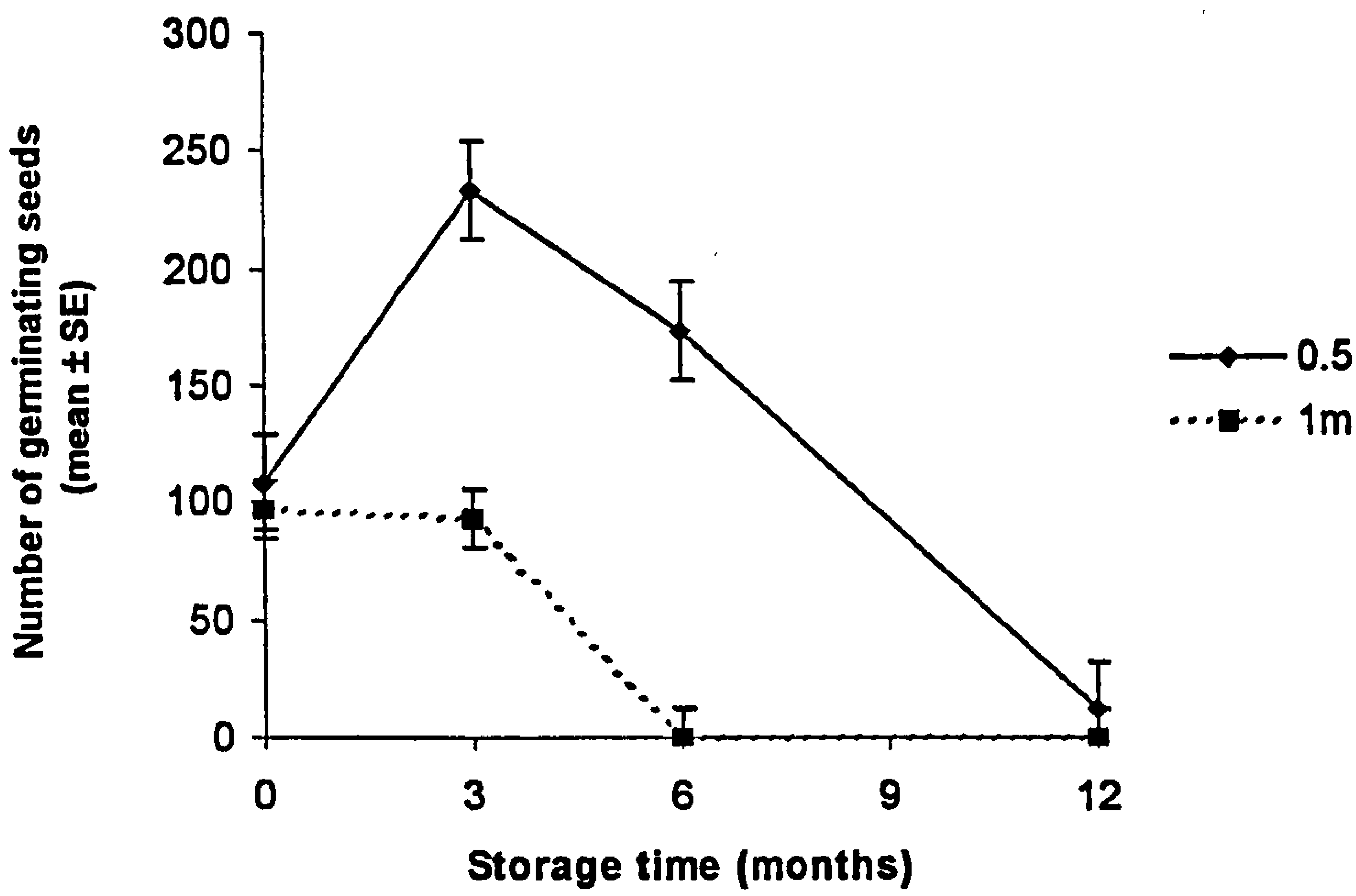


Figure 5.2 The influence of storage time on the germination of *Anthoxanthum odoratum* seeds at two different storage depths.

5.3.3 *Sanguisorba officinalis.*

Germination of *S. officinalis* seeds reduced following storage within a manure heap. Statistically this reduction was significant ($F_{3,39}$=45.54; P <0.001) The germination of seed in the control treatment was significantly greater than that of the other storage times. The length of the storage period did not alter germination significantly. As can be seen in Figure 5.3 all the reduction in germination caused by the rotting process of the manure occurred within the first three months.

Following 3 months storage within the manure heap *S. officinalis* seeds showed a significant ($F_{1,9}$=12.94; P=0.007) reduction in germination when stored at a depth of 1 m compared to when stored at a depth of 0.5 m. This reduction is shown in Figure 5.3.

Following 6 months storage within the manure heap a significant, $F_{1,9}$ =5.87; P=0.042, difference in germination was recorded between the seeds stored at 1 m and those stored at 0.5 m. This reduction in germination following the deeper storage is shown in Figure 5.3.

After twelve months storage in the manure heap a significant, $F_{1,9} = 37.79$; $P < 0.001$, reduction in germination was recorded when the seeds were stored further into the manure heap.

The interaction between storage time and storage depth for *S. officinalis* is shown in Figure 5.3. The figure shows that whilst the seeds stored more deeply within the manure heap had lower germination rates the difference was minimal and the pattern of germination reduction was the same for each depth.

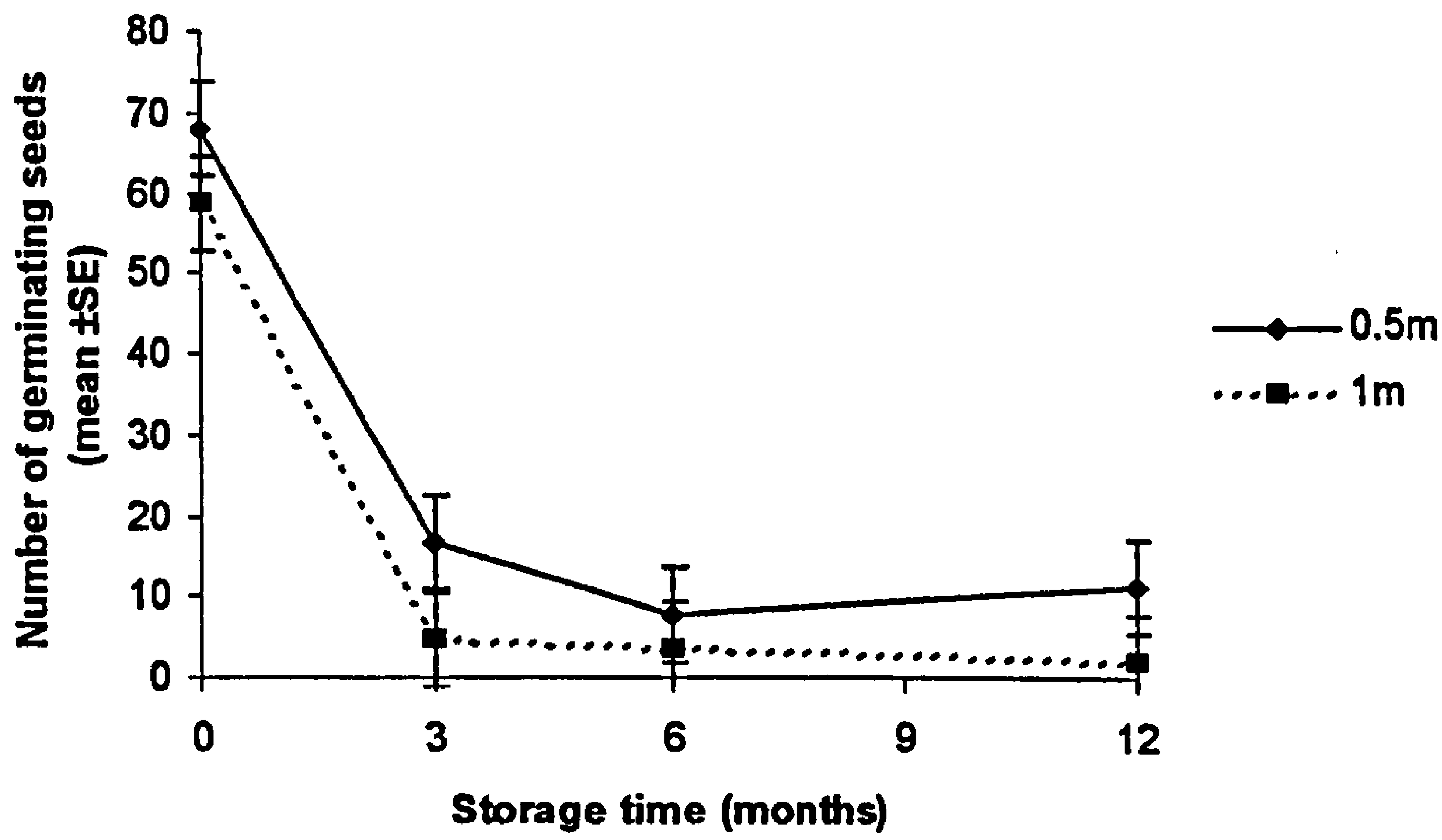


Figure 5.3 The influence of storage time on the germination of *Sanguisorba officinalis* seeds at two different storage depths.

5.3.4 *Filipendula ulmaria.*

F. ulmaria seeds when stored within the manure heap showed a significant $F_{3,39} = 53.92$; $P < 0.001$ reduction in germination as the length of storage increased. Initially 3 months storage produced a significant reduction in germination compared to the controls and there was a further significant reduction in germination between 3 and 6 months storage. This pattern, shown in Table Figure 5.4, was not repeated between 6 and 12 months storage which showed no significant difference in germination rate.

F. ulmaria seeds stored for three months within the manure heap showed a significantly, $F_{1,9} = 13.59$; $P < 0.001$, lower germination rate when stored at a depth of 1 m compared to those stored at a depth of 0.5 m.

After 6 months storage there was still a significant ($F_{1,9}=38.19$; $P < 0.001$) difference between the two depths of storage with the 1 m stored samples having a lower germination rate, see Figure 5.4.

Figure 5.4 shows the interaction of storage time or storage depth for *F. ulmaria* seeds. The reduction in germination bought about by 3 months storage time was steeper when the seeds were stored deeper within the manure heap. Following 6 and 12 months storage at a depth of 1 m germination was reduced to almost aero whereas at a storage depth of 0.5 m germination although much lower was occurring with some seeds.

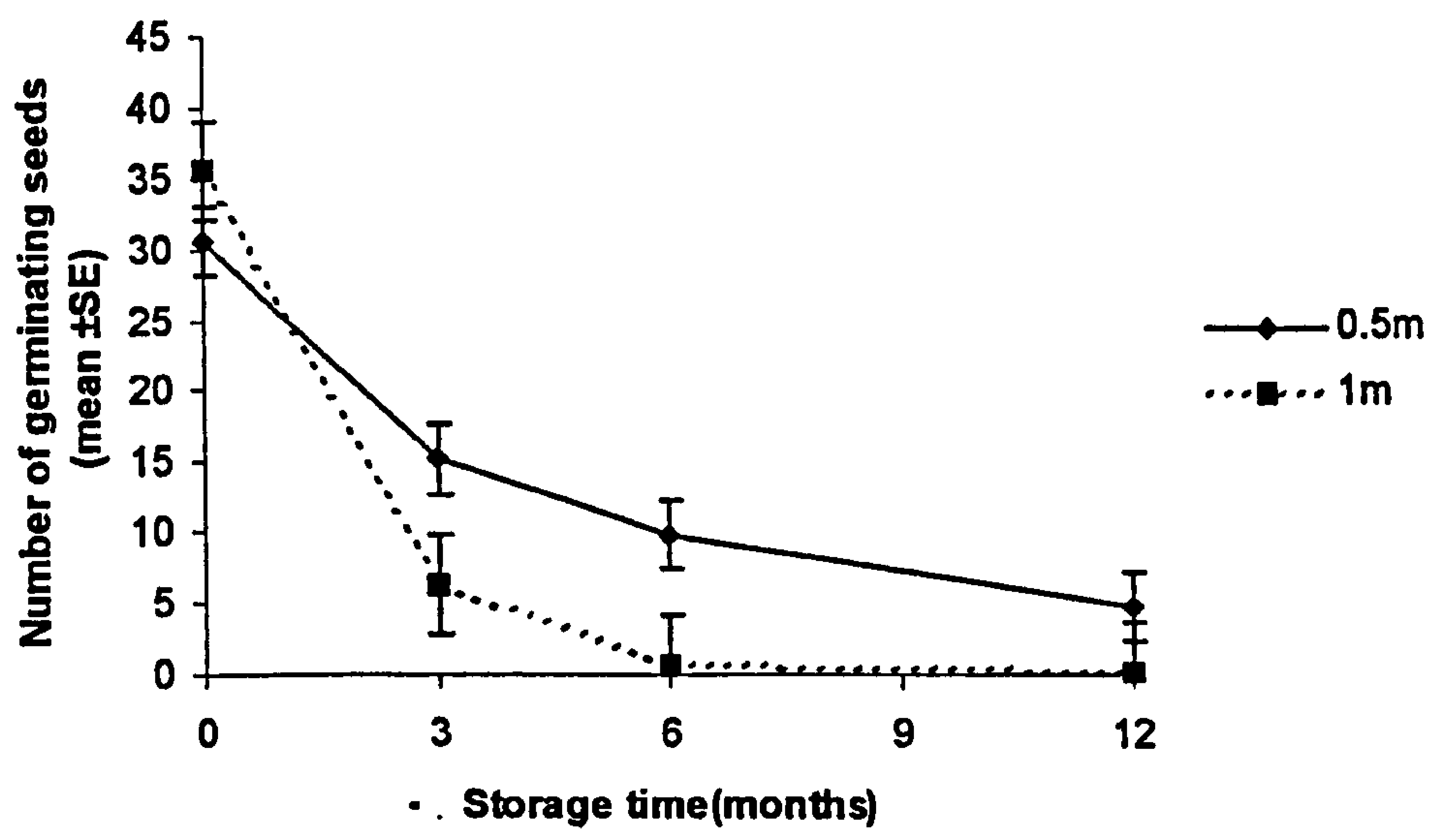


Figure 5.4 The influence of storage time on the germination of *Fillipendula ulmaria* seeds at two different storage depths.

5.3.5 *Myosotis arvensis.*

Storage of *M. arvensis* seeds within the manure heap gave rise to a significant reduction ($F_{1,19}$=23.13; P <0.001) in germination following three months. The extent of the reduction in germination is shown in Figure5.5. Following storage for 6 months and 12 months germination had been reduced to almost zero.

Following 3 months storage in a manure heap there was no significant difference ($F_{1,9}$=1.39; P=0.272) between seeds stored at 0.5 m and those stored at 1 m. Following storage for 6 and 12 months seed germination was reduced to almost zero at both depths as is shown in Figure 5.5.

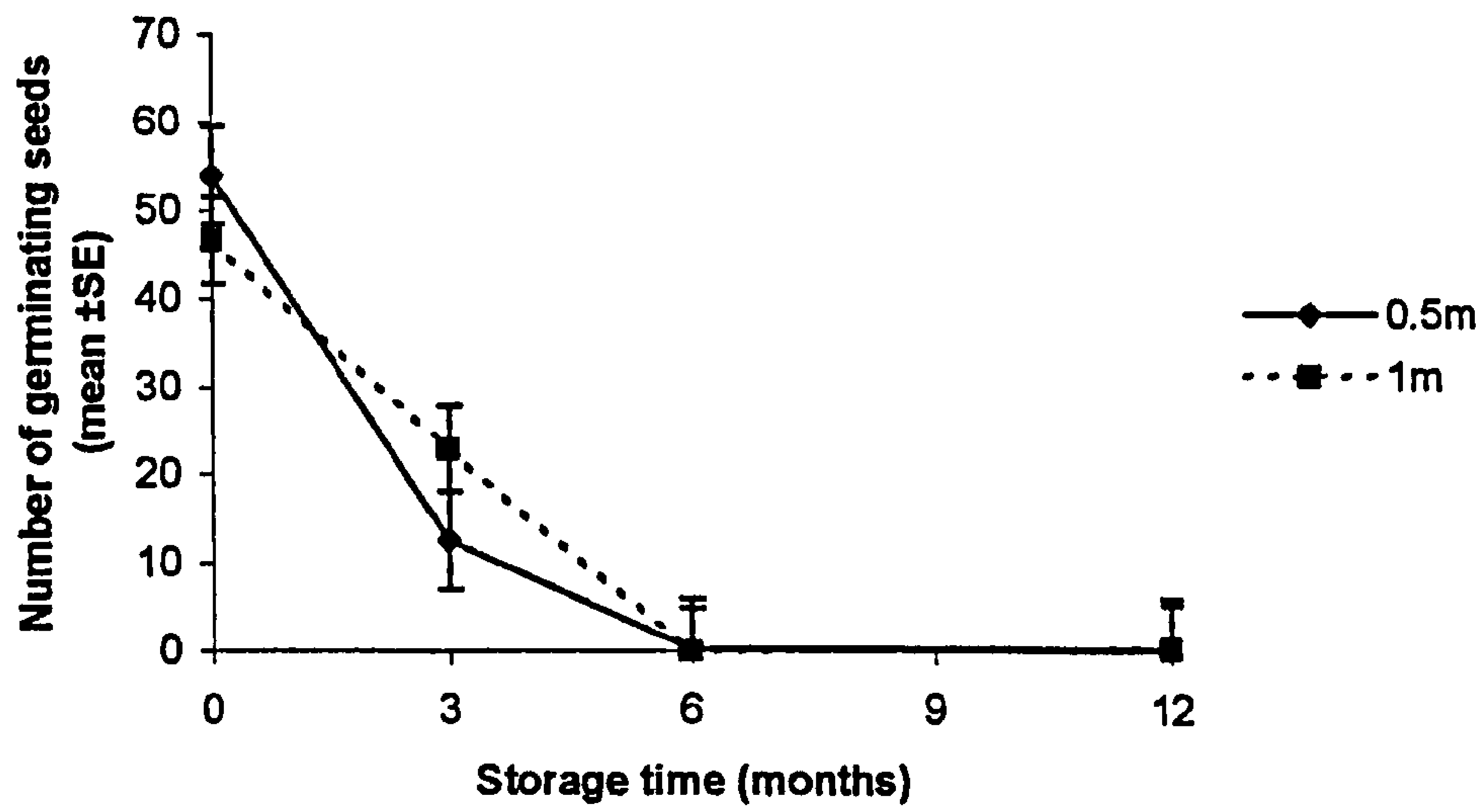


Figure 5.5 The influence of storage time on the germination of *Myosotis arvensis* seeds at two different storage depths.

5.4 Discussion.

The results achieved in this experiment demonstrate that some seeds of all the species tested here are capable of surviving for up to 12 months in a manure heap. However all species examined showed a reduction in germination rates following storage; these effects were not the same in each species. Viable seeds of the two species of grass tested in this experiment, *P. trivialis* and *A. odoratum* have been found in manure taken from traditionally managed meadow systems. Germination of *P. trivialis* was reduced following 3 months of storage. Six months storage further reduced germination but 12 months storage had no further effect. The depth at which the seeds were stored also had an influence; germination was lower when the seeds were stored deeper within the heap and this deeper storage increased the negative effect of storage time. It is suggested that some seeds of *P. trivialis* are resistant to the rotting process hence the lack of difference between 6 and 12 months storage.

The other grass species tested was *A. odoratum*. It was shown to be more resistant to rotting than *P. trivialis*. Following 3 months of storage at a depth of 0.5 m the seeds showed a significant rise in germination suggesting that the rotting process may have acted to break dormancy in the seeds. Subsequently only the 12 months storage time reduced germination significantly. Again with this species, the deeper the storage within the manure heap, the greater the negative effect of time on germination.

Two of the other species tested were the perennial dicotyledonous herbs *S. officinalis* and *F. ulmaria.* Previous chapters had determined that these species are largely absent from manure taken from traditionally managed meadow systems. *S officinalis* seeds reduced in germination rates following 3 months storage however this reduction was not continued in 6 and 12 months. This may suggest that a certain number of these seeds are capable of remaining viable within the manure heap. Those seed which were stored deeper within the manure heap were also reduced in viability and this deeper storage exacerbated the effects of storage time.

F. ulmaria seeds showed reductions in germination following 3 months storage which further reduced following 6 months. However 12 months storage did not further reduce germination rates. The depth at which the seeds were stored was also a significant factor in reducing germination. Those seeds stored deeper were less likely to germinate and, again, the depth of storage increased the effects of storage time.

It is possible therefore that these two species of dicotyledonous herb may remain viable within farmyard manure to be spread onto the meadow. This will nevertheless only occur if the hay cutting regime enables them to become incorporated into manure via the hay in the first place.

The only annual herb species tested was *M. arvensis*. This species showed very high sensitivity to storage within the manure heap. Depth of storage had no effect on germination rates as these were very low to begin with. Three months storage reduced germination rates to such a low level it was not possible to discern any further effects of storage time.

These results could also have implications for organic agriculture systems which are particularly vulnerable to weed infestations from manure spreading. Thill and Mallory-Smith (1997) suggested that farmers should be aware of the potential increase in weed problems associated with manure spreading. These results show however that storage of manure for longer periods will alleviate this problem and suggest periodic turning of the manure to improve composting would help reduce the weed dispersal capacity of the manure.

In general we can conclude that the seeds of these hay meadow plants are capable of remaining viable when stored in manure heaps. However when stored for 6-12 months this level of viability is likely to be very low. These results are similar to those reported by various workers, in general an increase in time of manure storage is associated with a reduction in the viability of seeds within the manure (Mt.Pleasant and Schlather 1994; Neto and Jones 1986; Remesova 2000). Those seeds stored deeper within the heap are also likely to be more reduced in germination capacity. This is most likely due to higher temperatures found deeper within the manure heap as these can reach 50°C (Rupende *et al.,* 1998). It is thought that as well as the action of heat on the seed the conversion of carbon dioxide, methane and ammonia to uric acid causes the seeds to rot (Poincelot 1975 in Rupende *et al.,* 1998). The temperatures within the heap are also at an optimum for micro-organisms to cause a rapid decomposition of the seed.

Whilst all species were reduced in germination, following 6 months storage, this reduction did not occur to the same extent and at the same time for each species. Farmyard manure could therefore play a role in the dispersal of viable seeds of some of the species which have become incorporated into it. However the lack of seed of species of particular conservation interest within hay and subsequently manure show that this particular route of dispersal whilst potentially being more important for species such as *P. trivialis* and *A. odoratum* may only occur for perennial dicotyledonous species such as *F. ulmaria* and *S. officinalis* in years of unusually late hay cuts when the possibility of seed becoming incorporated into the hay exists. The lack of germination of *M. arvensis* seeds suggest that manure spreading may not provide a suitable avenue for dispersal.

A. odoratum was the species most resistant to the rotting process of manure storage results which are consistent with the results from chapter 4 which showed that *A. odoratum* was more resistant to ruminant digestion than the other species tested here. *M. arvensis* in chapter 4 was seen as the least resistant to ruminant digestion which mirrors the results from this experiment. This suggests that the seed coat of *A. odoratum* is particularly resistant to break down and suggest that it is a species more likely to be dispersed by manure spreading than the others tested. The likely effects that seeds dispersal via manure spreading could have on the botanical composition of the meadow vegetation would therefore depend not only on the ability of other species not tested here to withstand this rotting process but also whether species of conservation interest such *Geranium sylvaticum,* in the case of northern hay meadows, become incorporated into the manure and this would depend on the timing of the hay cut.

6. Comparison of the soil seed bank of meadows with the vegetation and viable seed content of farmyard manure.

6.1 Introduction

In order to assess the role that farmyard manure could play as a source of seed within a traditionally managed hay meadow system it is necessary to gain some understanding of other sources of viable seed that could colonise gaps within the sward. One major source of germinable seed is the soil seed bank.

Soil seed banks have often been shown to differ from the vegetation which is growing above them (Kirkham and Kent 1997; Akinola *et al.,* 1998). This is due to a number of different factors such as differential seed production between species (Hodgson *et al.,* 1995) and differential germination and survival in the soil (Thompson *et al.,* 1997).

It is possible that seed may enter the soil seed bank of a meadow via a number of routes. Seed may be shed prior to the hay cut and during hay making operations (Smith *et al.,* 1996), or seed may disperse into the meadow from surrounding vegetation via wind dispersal. It has also been reported that seed may travel on the coats of stock (Fischer, Poschlod and Beinlich 1996) or on farm machinery (Strykstra *et al.,* 1996; Strykstra and Verweij 1997). The application of farmyard manure will also disperse a considerable quantity of seed into the meadow.

The purpose of this experiment was therefore to compare the seed banks in soil within the meadows of two traditionally managed farm systems to the vegetation in the meadows from which it was sampled, and then to compare the soil seed bank with the viable seed content of farmyard manure and hay. In order to aid the understanding of the differences between the meadow vegetation, soil seed bank and the viable seed contents of hay and manure the data were also compared to pseudo-random quadrats created with reference to various mesotrophic grassland sub-communities of the National Vegetation Classification (Rodwell 1992).

This experiment addresses the following questions.

1. Is the soil seed bank comparable to the meadow vegetation from which it originates?
2. How does the soil seed bank compare to the viable seed content of farmyard manure and could farmyard manure application play a role in the incorporation of seed into the soil seed bank?
3. Are those species of conservation interest within the meadows, which are absent from farmyard manure, found within the soil seed bank?

6.2 Methods.

The methods used in this experiment are an adaptation of those described by Thompson *et al.,* (1997) the main difference being in the timing of the soil collection. Their methods recommend soil collection in the spring in order to enable any seed chilling requirements to take place prior to germination. However in this experiment there is an interest in the transient as well as the permanent seed bank which necessitated a collection in late summer. This was done as soon after the hay cut as was possible. Any cold chilling requirements of the seeds was provided by the use of a non-heated glasshouse for the germination of the seed samples.

6.2.1 Soil Collection.

Soil samples were collected using a 12.5 cm by x 10 cm soil corer. Samples were collected from Piper Hole and New House Farm. The samples were collected during the early part of September 2000.

At Piper Hole three soil cores were taken at random from each of five meadows. At New House Farm three soil cores were taken at random from 4 meadows. Samples were then divided into half with the upper 5cm separated from the lower 5cm.

6.2.2 Bulk Reduction of Soil Samples.

Bulk reduction has been shown to increase the germination rate of seeds from soil samples and was also necessary in order to keep the amount of glasshouse space used reasonable. Ter Heerdt et al., (1996) showed that a soil sample that had been concentrated took 6 weeks for full germination to occur whereas similar non-concentrated samples took 4 to 6 months. Bulk reduction was done by washing the samples through various sizes of course sieves and removing stones and root material.

A fine 0.212 mm mesh sieve was used to collect the seeds and other fine particles. This size of mesh has been shown to be sufficiently small enough to ensure the retention seed of the majority of species including *Juncus* species, which have particularly small seeds (Thompson *et al.*, 1997).

6.2.3 Seed Germination.

Germination of the samples was achieved by spreading the reduced soil material thinly (less than 1 cm) onto seed trays containing sterile potting compost. The trays were then placed in an unheated glass house for a year. It has been shown that only seeds at the surface will germinate (Williams 1969). Seeds that are buried more deeply may be unable to germinate because of the reduction in light intensity (Fenner 1985). In order to overcome this problem following periods in which no germination took place the samples were thoroughly mixed.

Germinating seeds were identified and removed weekly. A number of species were only identified to the following groups; *Ranunculus repens*, *R. bulbosus* and *R. acris* were all recorded as *Ranunculus* spp. and *Trifolium repens* and *T. pratense* were recorded as *Trifolium* spp. Seedlings requiring continued growth to allow accurate identification were re-potted and grown on separately.

6.2.4 Data Analysis.

Paired t-tests were used to compare the number of seeds germinating from the upper 5 cm and lower 5 cm fractions of the soil cores using Minitab for Windows version 13.

The species composition of the soil seed bank and the viable seed content of the farmyard manure samples were compared with the vegetation from which they originated using Principal Component Analysis (PCA) plots of percentage occurrence, in the seed bank and manure samples, with percentage cover data from the vegetation. The PCA ordination was completed using Canoco (version 4) (ter Braak and Smilauer 1998).

The analysis also included the use of data relating to the National Vegetation Classification (NVC) (Rodwell 1992). Following the method of Smith *et al.*, (2002) the data published within the NVC were used to generate simulated sub-community data in the form of pseudo-random quadrats from various mesotrophic grassland sub-communities related to MG3 (for the full list see Appendix 9). The data used were representative of typical sub-community quadrats, with an appropriate species composition as well as frequency and abundance. Pseudo random quadrats were generated by initially choosing a species at random from the full floristics table from that sub-community as published in Rodwell (1992). This choice was based on the frequency with which it is found within the sub-community so that those species which are constant within the sub-community (frequency score of 5) were automatically chosen. Next, the Domin value scores of abundance were converted to percentage cover using the method of Curral (1987) and a random cover value chosen for each selected species. The process was halted when, for each quadrat, the total number of species was approximately equal to the mean number of species published in the NVC. In total 50 quadrats were produced for each sub-community.

The pseudo-random quadrat data were then analysed using PCA. The PCA ordination was completed using Canoco (version 4) (ter Braak and Smilauer 1998). The data from each meadow system relating to the soil seed bank, the seed content of hay and manure as well as the meadow vegetation itself were added to the analysis as supplementary data. Supplementary data, also known as passive data, do not influence the ordination axes. It is therefore possible to judge the relationships between the supplementary data and the active data which is in this case the mean positions of pseudo quadrats containing various NVC mesotrophic grassland sub-communities. Prior to the analysis, the NVC pseudo-random quadrat data for *Ranunculus acris, R. bulbosus* and *R. repens* were combined to form *Ranunculus spp.* as were the *Alchemilla* species to form *A. vulgaris* agg. and *Trifolium repens* and *Trifolium pratense* were combined to form *Trifolium spp.* This was done in order to bring the NVC data in line with the other data sets.

6.3 Results.

6.3.1 Comparison of Piper Hole Meadows Vegetation with the Soil Seed Bank.

The mean number of species in the upper fraction of the soil cores was significantly higher than the number of species in the lower fraction of the soil core as is shown in Table 6.1. *Poa trivialis* was the most common species found in the soil samples. Other more commonly occuring species found were the grasses *Alopecurus pratense, Anthoxanthum odoratum, Agrostis capillaris, Bromus hordeaceus, Lolium perenne* and *Holcus lanatus* whilst common species of dicotyledonous herbs *included Myosotis discolor, Ranunculus* spp., *Plantago lanceolata, Cerastium fontanum, Rhinanthus minor, Alchemilla vulgaris* agg. and *Bellis perennis.* Of these species only the early flowering herbs *R. minor* and *A vulgaris* agg. are species of conservation value. The later flowering perennial herbs present in the vegetation of particular conservation value such as *Sanguisorba officinalis, Geranium sylvaticum, Geranium pratense, Filipendula ulmaria* and *Cirsium helenioides* were either absent or present in very low numbers.

Table 6.1 The mean number of germinating seeds from all 15 12.5 cm^2 soil cores (total area 187.5 cm^2) and percentage frequency in both layers of the soil cores from meadows at Piper Hole. Results of Paired T-Test of the mean number of germinating seeds of each species between the two layers of the soil core are also given. Only species occurring at a frequency greater than 20% are shown, the remaining species are listed in Appendix 10.

	0-5cm soil layer		6-10 cm soil layer		Paired T- Test	
	Percentage Frequency	Mean	Percentage Frequency	Mean	T	P
Mean number of species in soil core.		18.9		9.8	6.71	<0.001
Poa trivialis	100	48.4	78.6	8.0	4.22	0.001
Myosotis discolor	100	21.6	64.3	3.1	4.60	<0.001
Ranunculus spp.	100	36.0	100	4.7	5.15	<0.001
Alopecurus pratense	92.9	5.9	42.9	0.6	1.67	<0.001
Plantago lanceolata	92.9	5.6	21.4	0.2	4.19	0.001
Anthoxanthum odoratum	92.9	8.0	28.6	0.5	5.95	<0.001
Cerastium fontanum	92.9	7.7	57.1	1.9	4.17	0.001
Rhinanthus minor	92.9	7.4	14.3	0.1	2.62	0.021
Agrostis capillaris	85.7	2.4	21.4	0.4	4.60	<0.001
Alchemilla vulgaris agg.	85.7	10.9	92.9	14.0	-0.70	0.497
Bromus hordeaceus	78.6	6.1	7.1	0.2	3.52	0.004
Bellis perennis	78.6	6.8	14.3	0.4	4.69	<0.001
Lolium perenne	71.4	2.8	0.0	0.0	4.42	0.001
Rumex acetosa	71.4	2.9	7.1	0.1	2.69	0.019
Holcus lanatus	57.1	1.9	21.4	0.3	1.67	0.119
Juncus bufonius	57.1	0.9	57.1	1.5	-0.72	0.486
Trifolium spp.	57.1	1.3	21.4	0.3	1.19	0.079
Montia fontanum	50.0	10.6	21.4	2.6	-1.07	0.304
Veronica serpyllifolia	50.0	3.4	78.6	3.0	0.37	0.718
Poa pratensis	42.9	1.2	14.3	0.2	1.90	0.082
Phleum pratense	42.9	1.1	28.6	0.4	0.81	0.435

Cardamine pratensis	42.9	2.6	28.6	3.0	-0.55	0.593
Anthriscus sylvestris	35.7	1.0	14.3	0.1	1.79	0.097
Juncus effusus	28.6	0.4	42.9	0.8	-1.25	0.234
Stellaria media	28.6	0.9	7.1	0.1	1.63	0.127
Cirsium arvensis	21.4	0.3	0.0	0.0	1.75	0.104
Geranium sylvaticum	21.4	0.4	0.0	0.0	1.79	0.096

Whilst no species were significantly more common in the lower fraction of the soil a few of the more common species such as *A. vulgaris* agg. and the rushes *Juncus bufonius* and *Juncus effusus* were more frequently found in the lower fraction of the soil.

Figure 6.1a, the PCA plot of Piper Hole soil seed bank samples and meadow vegetation samples, shows the meadow vegetation as being in a tight cluster on the left of the graph whilst the soil seed bank samples are more variable and to the right of the vegetation samples forming a separate group. In general the soil seed bank samples from the lower part of the soil core form the upper part of the seed bank group, in the upper right hand side of the graph and the seed bank samples from the upper part of the soil core are found in the bottom right side of the graph.

Comparison of Figure 6.1a with 6.1b the PCA plot of Piper Hole seed bank species and meadow vegetation species shows that the cluster of samples representing the meadow vegetation is typified by species including the perennial herbs *G. sylvaticum*, *F. ulmaria* and *S. officinalis*. The upper right side of the species plot (Figure 1b) shows a strong relationship with *A. vulgaris* agg. and the samples from the lower part of the soil core. The rush species *J. effuses* and *J. bufonius* are also shown as being more common in the lower part of the soil core.

The lower right side of Figure 6.1b shows that *P. trivialis* and *Ranunculus* spp. are strongly associated with the soil samples from the top of the soil core. Other species such as the annual herbs *Myosotis* spp. *Cerastium fontanum* and *Montia fontanum* as well as species of grass such as *Agrostis capillaris* and *Alopecurus pratensis* are also common within the top layer of the soil core.

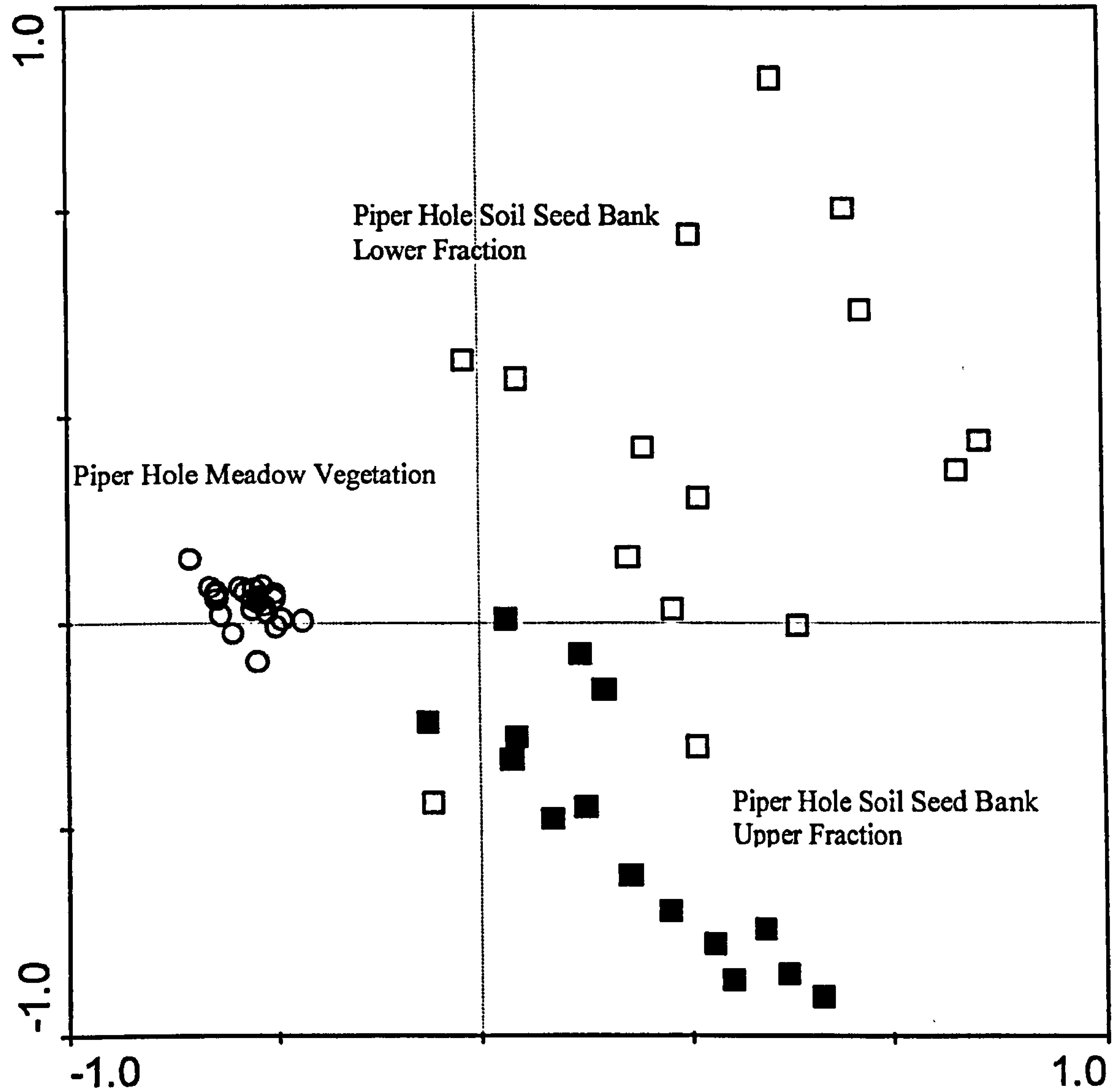
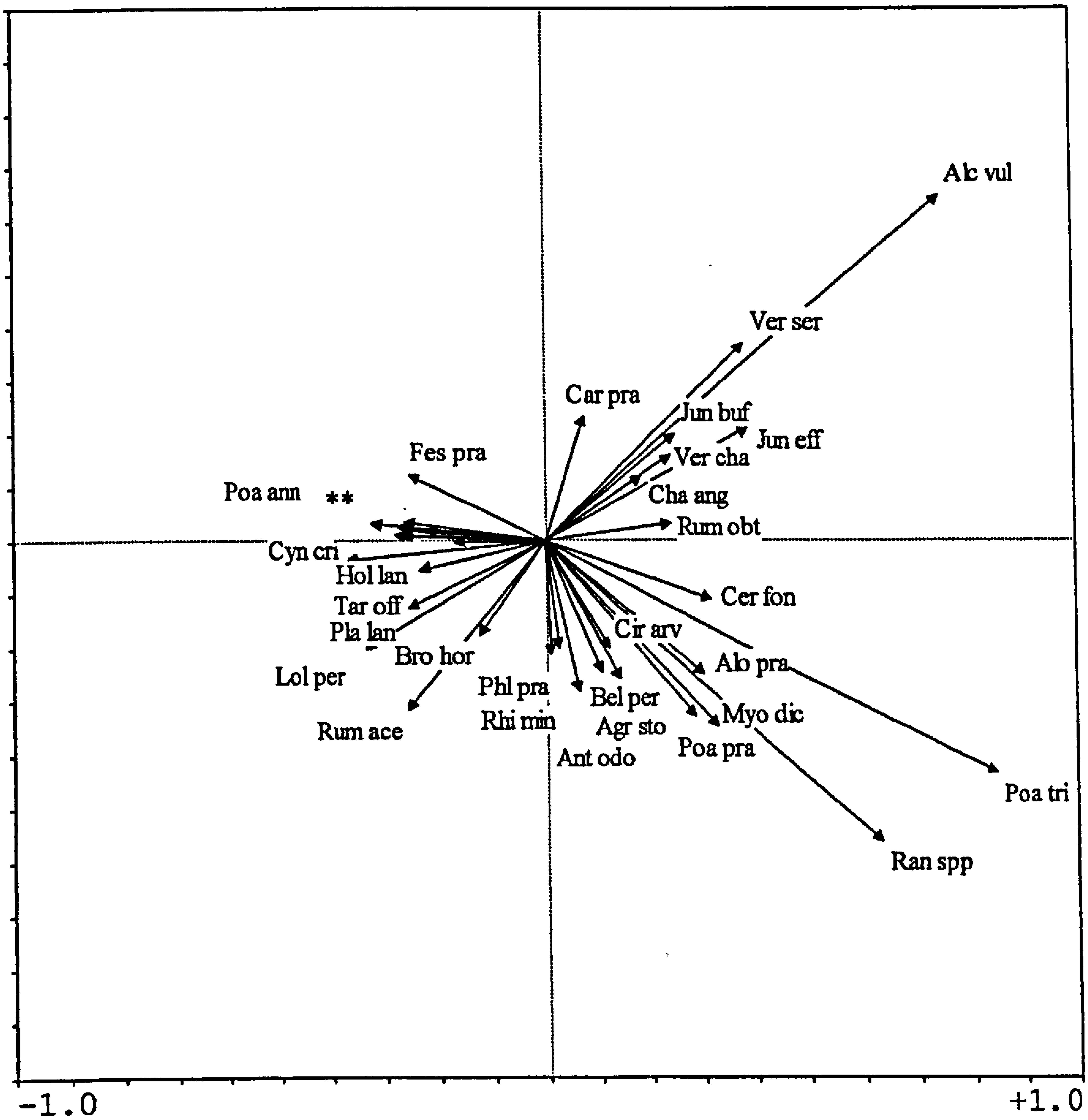


Figure 6.1a. PCA plot showing the site ordination of Piper Hole soil and vegetation samples ■upper fraction samples, □ lower fraction samples, O meadow vegetation samples.



** San off, Ger syl, Fil ulm, Con maj, Lat pra, Vic sat and Poa ann

Figure 6.1b The PCA plot showing the species ordination of Piper Hole soil and vegetation samples.

As is shown in Table 6.1 both the diversity of species and the total number of seeds was greater in the upper part of the soil cores than in the lower part. The upper part of the soil core is more likely to provide propagules to any gaps created in meadow vegetation and so in order to look more closely at the comparison between the upper part of the soil core and the meadow vegetation, PCA was carried out omitting the samples from the lower part of the soil core.

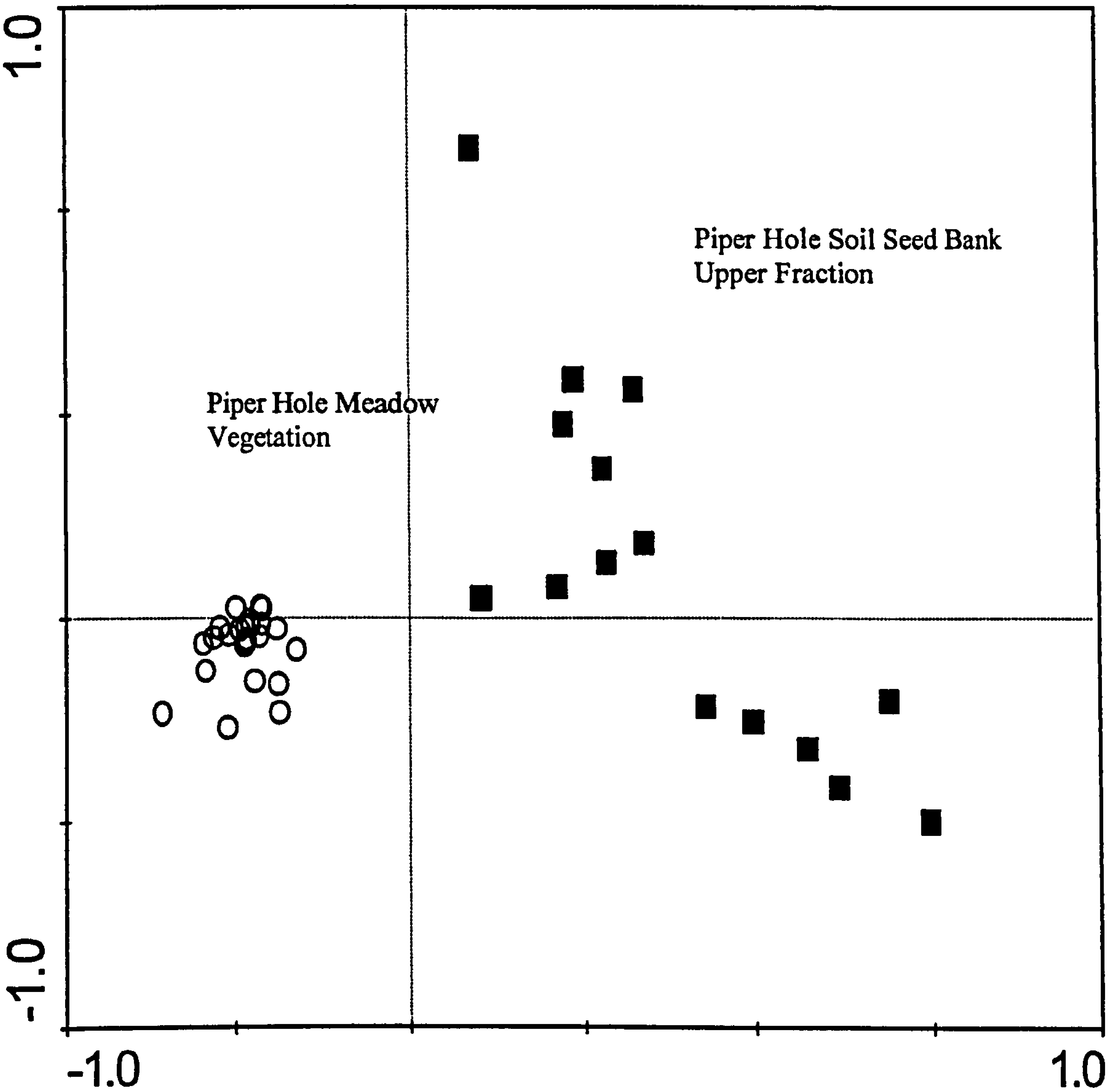


Figure 6.2a. PCA plot showing the site ordination of Piper Hole soil upper 5cm

fraction only and vegetation samples. □ upper fraction samples, meadow vegetation samples●.

Figure 6.2a the PCA plot of the upper fraction of the soil core samples and the meadow vegetation samples shows the meadow vegetation grouped together on the left of the graph whereas the soil seed bank samples form two distinct groups those in the upper right of the graph and those on the lower right of the graph. One of the soil seed bank samples is found towards the top of the graph on its own.

The group of 4 seed bank samples found on the bottom right of Figure 6.2a are as is shown in Figure 6.2b dominated by *P. trivialis* with *P. pratensis* and *Agrostis capillaris* also prominent. These samples are not as diverse as the soil samples grouped together on the top right of Figure 6.2a. These samples are characterised by species of annual herb such as *Myosotis* spp., *Cerastium fontanum* and to a lesser extent *Rhinanthus minor* as well as the perennials *Ranunculus* spp., *Alchemilla vulgaris* agg.

The reasons for the different clusters of the upper fraction of the soil seed bank are unclear.

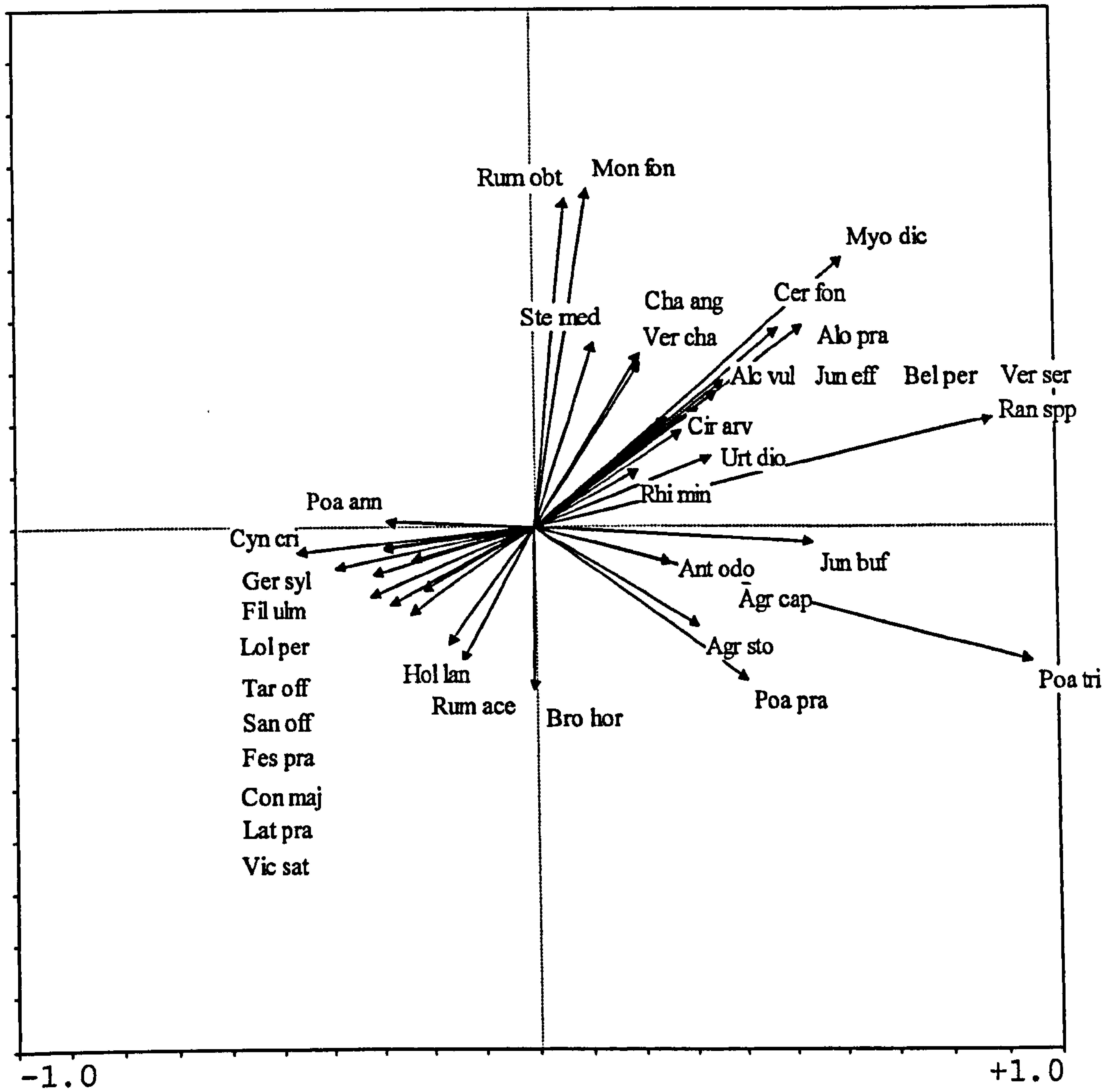


Figure 6.2b PCA plot showing the species ordination of Piper Hole soil upper 5cm fraction only and vegetation samples.

6.4.2 Comparison of New House Farm Meadows Vegetation with the Soil Seed Bank

As can be seen in Table 6.2 the soils from the upper part of the cores from New House Farm contained significantly more species of viable seed than the lower part of the soil core they also contained more viable seed in total. The majority of species were recorded in greater numbers and at a greater frequency in the upper part of the soil cores. As is shown in Table 6.2 the majority of species found were grasses such as *P. trivialis*, *H. lanatus*, and *A. odoratum*. Annual dicotyledonous herbs such as *Myosotis* spp. and *C. fontanum* were also very prominent in these soil samples as were perennial herbs such as *P. lanceolata* and *Ranunculus* spp. The annual herbs of conservation interest *R. minor* and *A. vulgaris* agg. were less common in the New House samples than the Piper Hole samples, whilst the perennial herbs such as *G. sylvaticum* and *S. officinalis* were entirely absent.

As was observed in the Piper Hole seed bank, no species were significantly more common in the lower fraction of the soil core. *A. vulgaris* agg. however, was found more often in the lower fraction of the soil.

Table 6.2 The mean number of germinating seeds from all 12 12.5 cm^2 soil cores (total area 150 cm^2) and percentage frequency in both layers of the soil cores from meadows at New House Farm Results of Paired T-Test of the mean number of germinating seeds of each species between the two layers of the soil core are also given. Only species occurring at a frequency greater than 20% are shown, the remaining species are listed in Appendix 11.

	0-5cm soil layer		6-10 cm soil layer		Paired T- Test	
	Percentage Frequency	Mean	Percentage Frequency	Mean	T	P
Number of species in soil core		13.92		7.75	6.22	<0.001
Poa trivialis	100	24.58	75	4.75	3.58	0.004
Myosotis spp.	100.00	11.08	41.67	0.58	3.83	0.003
Cerastium fontanum	100.00	13.25	66.67	2.75	3.71	0.003
Bellis perennis	91.67	49.83	91.67	3.75	5.63	<0.001
Plantago lanceolata	91.67	7.50	25.00	0.33	4.14	0.002
Ranunculus spp.	91.67	10.67	66.67	1.75	4.01	0.002
Juncus bufonius	83.33	1.333	41.67	0.917	0.63	0.539
Juncus effusus	66.67	1.250	33.33	0.917	1.00	0.339
Trifolium sp	58.33	1.833	16.67	0.167	2.42	0.034
Anthoxanthum odoratum	58.33	2.091	8.33	0.091	2.58	0.027
Taraxacum officinale agg.	50.00	1.000	8.33	0.083	2.56	0.026
Holcus lanatus	50.00	1.417	16.67	0.667	1.09	0.298
Bromus hordeaceus	41.67	3.08	0	0.00	1.80	0.100
Poa pratense	41.67	1.083	0	0.00	2.31	0.041
Alchemilla vulgaris agg.	33.33	1.42	50.00	2.83	-0.66	0.524
Alopecurus pratense	33.33	1.231	16.67	0.389	1.25	0.236
Cardamine pratensis	33.33	0.500	0.00	0.000	1.91	0.082
Veronica serpyllifolia	33.33	1.583	58.33	1.583	0.00	1.000

Figure 6.3a the PCA plot of the New House Farm meadow vegetation and soil seed bank (both upper and lower fractions of the soil core) shows the meadow vegetation on the right of the graph with the exception of one sample and the soil seed bank samples on the left of the diagram. The meadow vegetation at New House Farm is as variable as the soil seed bank samples, this is in contrast to what was seen with the Piper Hole samples in Figure 6.1a.

The upper left part of Figure 6.3a is the part of the graph which mainly contains the soil samples from the lower fraction of the soil core whereas the lower left section of the graph contains those soil samples which originated from the upper part of the soil core.

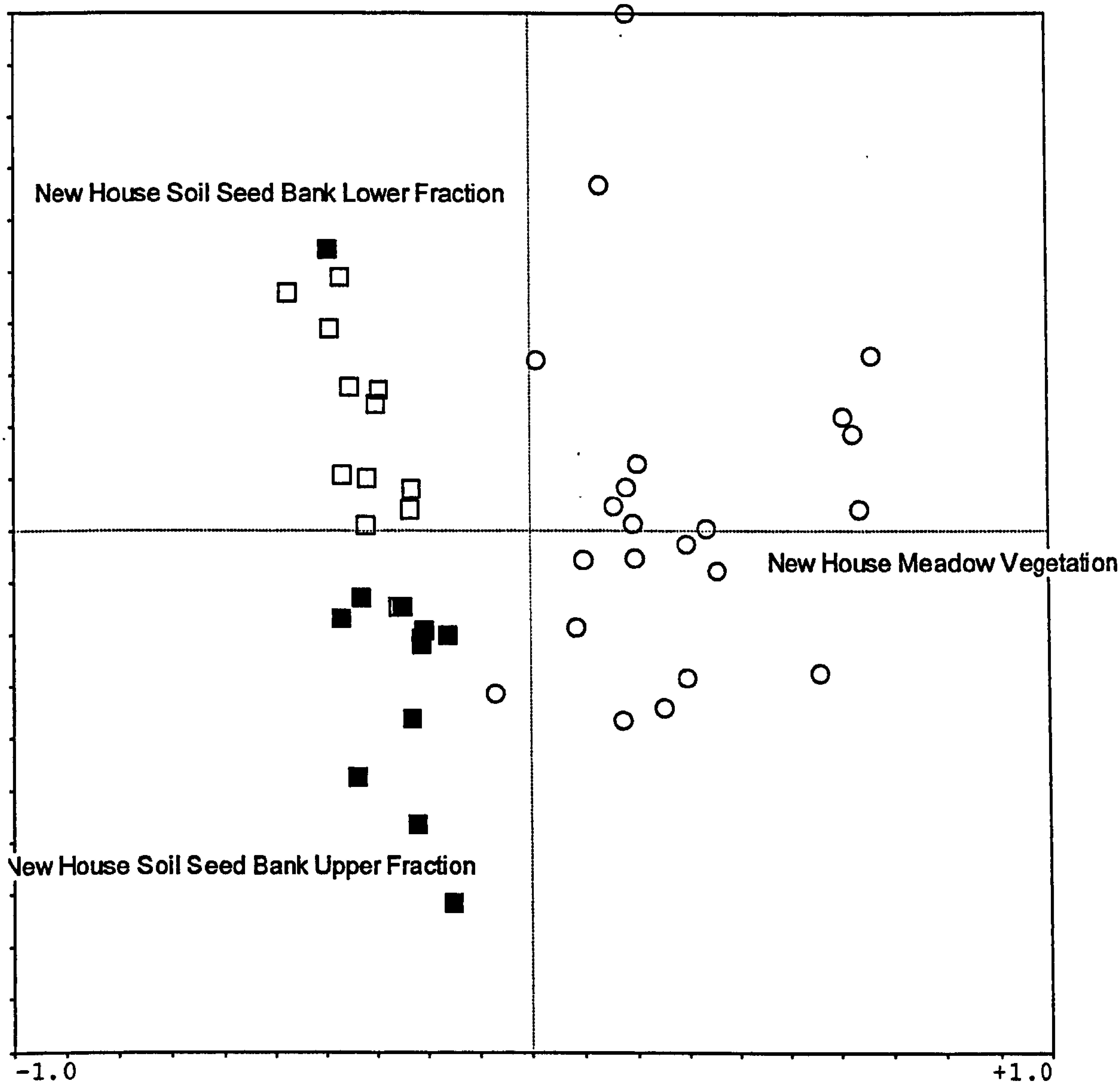
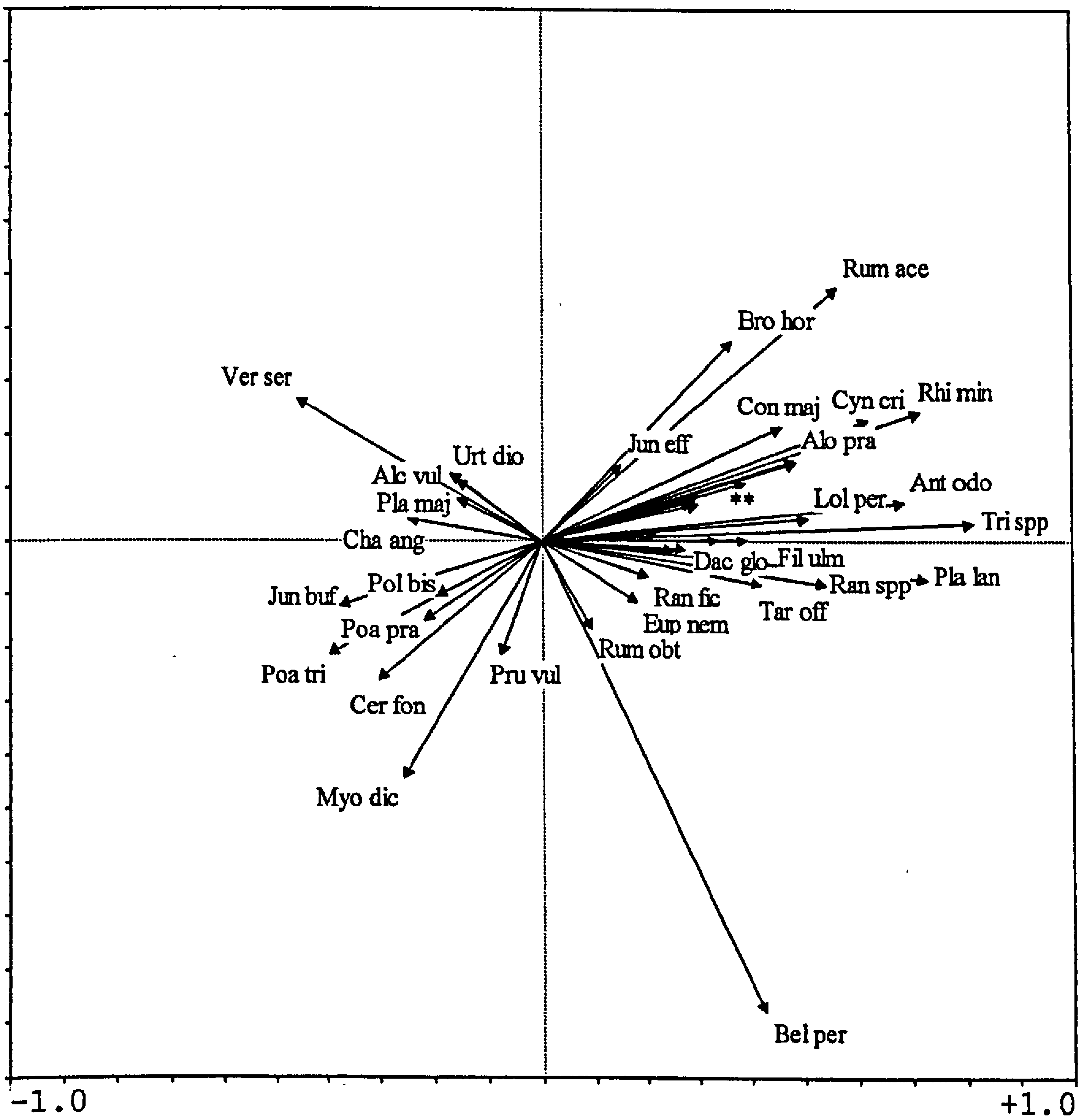


Figure 6.3a. PCA plot showing the site ordination of New House Farm soil and vegetation samples ■ upper fraction samples, □ lower fraction samples, ● meadow vegetation samples.

Figure 6.3b the species plot of the soil seed bank and meadow vegetation at New House Farm shows once again that the meadow vegetation is characterised by perennial herbs such as *S. officinalis, G. sylvaticum* and *Caltha palustris,* whereas the soil seed bank is dominated by the grass *P. trivialis* and the annual herbs

Myosotis sp and *C. fontanum* with the rush *Juncus bufonius* also prominent. This is a similar pattern to that seen at Piper Hole.



** Phl pra, Ant syl, Cen nig, Fes rub, Ste med, Fes pra and Ger syl.

Figure 6.3b The PCA plot showing the species ordination of New House soil and vegetation samples.

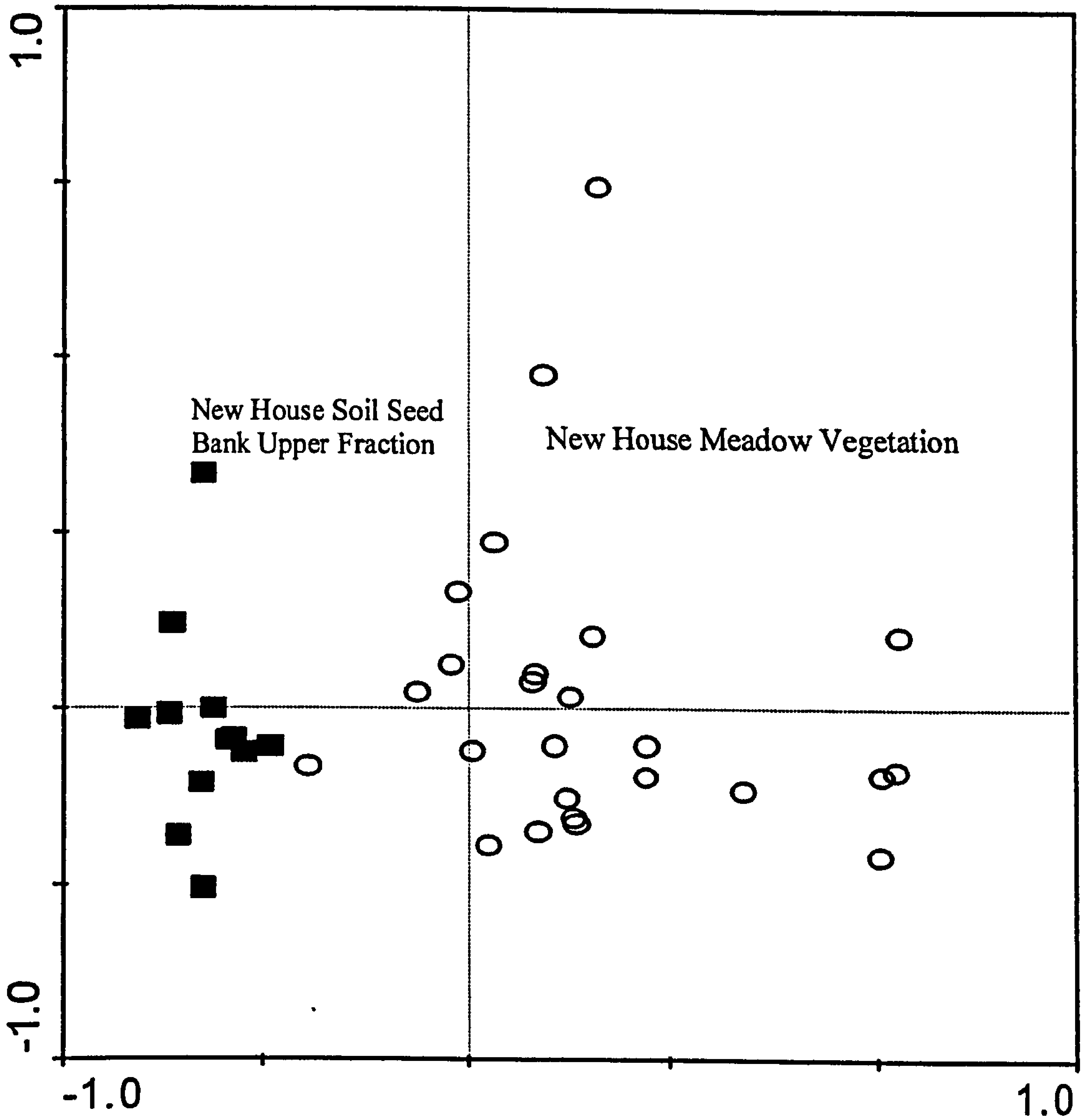


Figure 6.4a PCA plot showing the site ordination of New House Farm soil upper 5cm fraction only and vegetation samples. ■upper fraction soil samples, meadow vegetation samplesO.

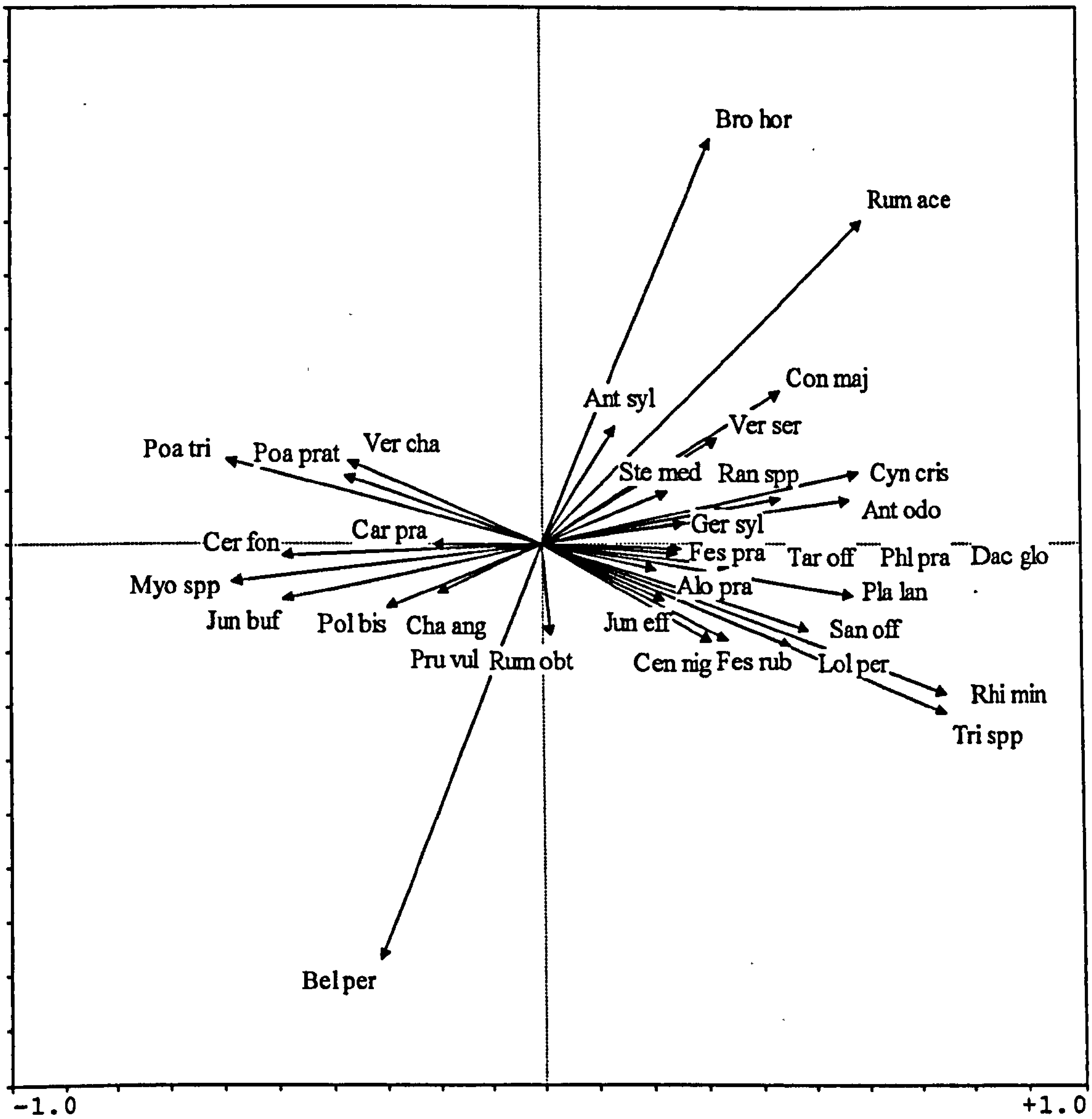


Figure 6.4b PCA plot showing the species ordination of New House soil upper fraction and meadow vegetation.

Figure 6.4a the PCA plot of the upper fraction of the soil core and the meadow vegetation samples shows the meadow vegetation and soil seed bank as two groups, although these groups are less distinct than is the case with the Piper Hole samples in Figure 2a. The meadow vegetation samples are show to the right of the soil seed bank samples with both groups showing a similar amount of variability.

Comparison of Figure 6.4a and 6.4b shows that *Bellis perennis, Poa trivialis* and the annual herbs *Cerastium fontanum* and *Myosotis* sp are characteristic of the upper fraction of the soil cores compared to the meadow vegetation. The meadow vegetation is characterised by *Rhinanthus minor* in contrast to the situation with the samples from Piper Hole. Perennial herbs such as *Geranium sylvaticum* and *Sanguisorba officinalis* are also more common in the meadows than in the upper fraction of the meadows soil seed bank.

6.4.3 Comparison of Piper Hole Vegetation with the Soil Seed Bank and the Viable Seed Content of Farmyard Manure and Hay Samples.

Table 6.3 shows the differences in species composition of the Piper Hole meadow vegetation, the upper fraction of the soil core, hay samples and manure samples of various ages. It is clear from this Table that differences in the species composition occur. The inclusion of data of the viable seed contents of hay and manure samples from Piper Hole Meadows with the vegetation and soil seed bank within the PCA analysis is shown in Figures 6.5a and 6.5b. From Figure 6.5a, it is clear that in general the hay and manure samples are grouped separately from the soil seed bank samples which in turn differ from the meadow vegetation.

The meadow vegetation samples form a reasonably tight group in the lower left part of the graph with the soil seed bank samples forming a group mostly found within the upper left part of the graph. The soil seed bank group mainly varies along the y axis of the graph. The hay and manure samples with the exception of hay sample number 4 occur along the x axis of the graph on the right.

Table 6.3 The frequency and domin value range of Piper Hole Meadow vegetation and the mean number of germinating seed and percentage frequency in the upper fraction of the soil core, hay samples and manure samples of various ages.

Species	Piper Hole Vegetation		Piper Hole Soil Seed Bank		Piper Hole Hay		Piper Hole Manure	
	Percentage frequency	Domin Value range.	Mean seed	Percentage frequency	Mean seed	Percentage frequency	Mean seed	Percentage frequency
Anthoxanthum odoratum	100.0	2-4	8.0	92.9	14.2	100	0.6	25
Holcus lanatus	100.0	1-6	1.9	57.1	35.6	100	0.2	25
Lolium perenne	95.8	1-4	2.8	71.4	156.2	100	16.5	75
Bromus hordeaceus	95.8	1-5	6.1	78.6	11.4	80	0.7	37.5
Plantago lanceolata	87.5	1-5	5.6	92.9	32.4	80	1.2	62.5
Rumex acetosa	83.3	1-4	2.9	71.4	8.6	100	0.5	50
Ranunculus spp.	83.3	1-4	36.0	100	5.4	60	0.9	50
Bellis perennis	82.5	1-4	6.8	78.6	8.2	100		
Cynosurus cristatus	70.8	1-4			3.6	60		
Poa trivialis	70.8	1-4	48.4	100	773	100	99.2	100
Trifolium spp.	66.7	1-5	1.3	57.1	0.4	40	0.5	50
Sanguisorba officinalis	62.5	2-6						
Rhinanthus minor	62.5	1-4	7.4	92.9				
Agrostis capillaris	58.3	1-3	2.4	85.7	50.6	100	0.4	37.5
Myosotis spp.		1-3	21.6	100	16.8	100	1.1	50
Cerastium fontanum	58.3	1-3	7.7	92.9	9.4	100	0.3	25
Taraxacum officinale agg.	58.3	1-5					0.1	12.5
Anthriscus sylvestris	54.2	1-5	1.0	35.7				
Filipendula ulmaria	54.2	1-5						
Geranium sylvaticum	45.8	1-6	0.4	21.4				
Phleum pratense	41.7	1-3	1.1	42.9	42.6	100	0.3	37.5
Poa annua	37.5	1-5			3.8	60		
Festuca pratensis	33.3	1-2			0.4	40	0.1	12.5
Vicia sativa	29.2	1-4						
Lathyrus pratensis	25.0	1						
Alopecurus pratensis	20.8	1	5.9	92.9	15.2	100	0.2	12.5
Conopodium majus	20.8	1						
Geranium pratense	16.7	2-5						

Poa pratense	16.7	1			107.8	100	6.0	62.5
Dactylis glomerata	16.7	1			122	100	0.5	25
Arrhenatherum elatius	12.5	1-2			2	40		
Alchemilla vulgaris agg.	12.5	1-3	10.9	85.7				
Heracleum sphondylium	12.5	1-4						
Stellaria media	12.5	1-3	0.9	28.6	0.4	40	0.1	12.5
Cirsium helenioides	12.5	2-4						
Euphrasia nemorosa	12.5	1						
Centaurea nigra	8.3	1-6						
Caltha palustris	8.3	5						
Juncus bufonius			0.9	57.1	1.6	40	2.2	62.5
Montia fontanum			10.6	50.0	2.8	40	0.1	12.5
Veronica serpyllifolia			3.4	50.0				
Cardamine pratensis			2.6	42.9				
Juncus effusus			0.4	28.6	3.4	80	0.4	37.5
Cirsium arvensis			0.3	21.4			0.1	12.5
Chamerion angustifolium					0.2	20	0.2	12.5
Deschampsia cespitosa					2.6	40		
Agrostis stolonifera					0.8	20		
Festuca arundinacea					0.2	20		
Polygonum persicaria					3.8	60		
Polygonum bistorta					2	100		
Ajuga reptans					0.4	40		
Urtica dioica					6	20		
Veronica chamaedrys					0.2	20		
Cirsium helenioides					0.4	20		
Vicia sepium					0.4	20		
Hordeum vulgare							0.4	25
Poa annua							0.1	12.5

Comparison of Figure 6.5a and Figure 6.5b shows that the grasses *P. trivialis* and *L. perenne* as well as the rush *J. bufonius* are more frequent within hay and manure samples, whereas species such as the early flowering dicotyledonous herbs *B.*

perennis, R. minor, Cerastium fontanum, Montia fontanum and *A. vulgaris* agg. are characteristic of the soil seed bank samples. Larger later flowering herbs such as *G. sylvaticum, F. ulmaria* and *S. officinalis* are seen more frequently within the meadow vegetation than as viable seed in either hay or manure and the soil seed bank.

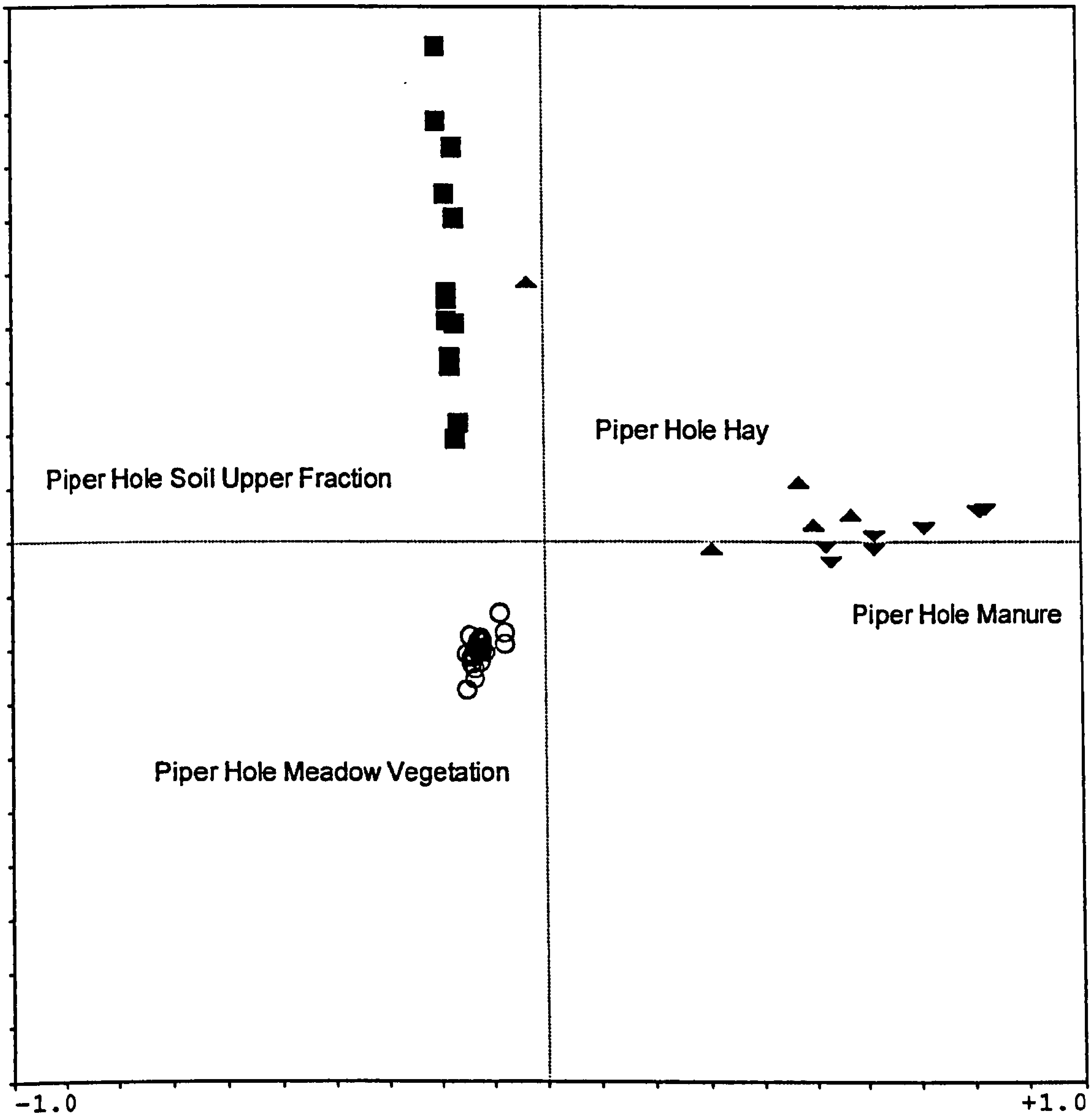
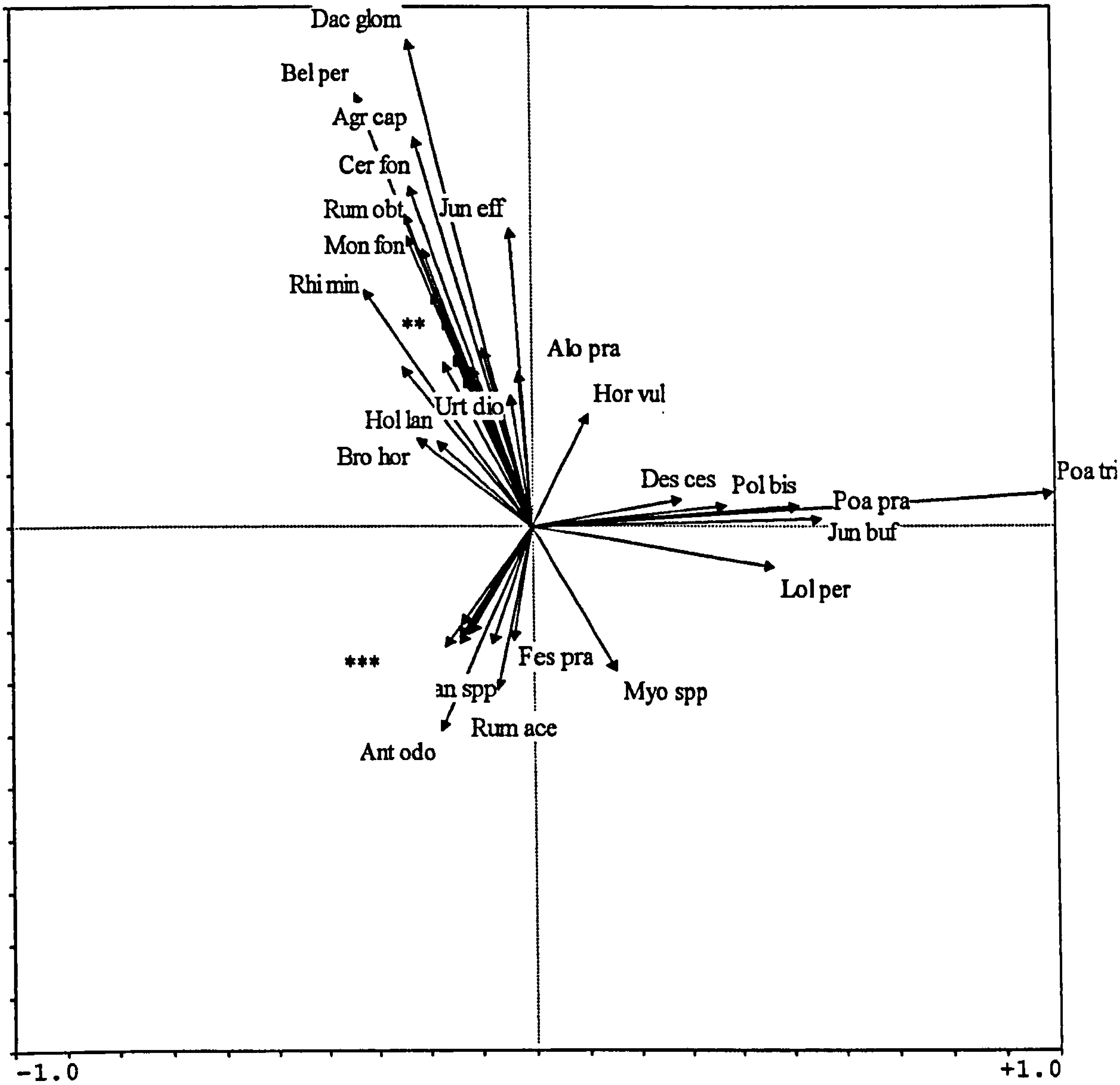


Figure 6.5a PCA plot showing the site ordination of Piper Hole △soil seed bank samples, ●hay and ▽manure samples and ■meadow vegetation samples.



** Cyn cris, Cirs arv, Alc vul, Vic sep, Vic cra, Poa ann and Pla lan

*** Ger syl, San off, Lat pra, Con maj, Fil ulm and Tar off.

Figure 6.5b PCA plot showing the species ordination of Piper Hole soil seed bank, hay, manure and meadow vegetation.

6.4.4 Comparison of New House Farm Vegetation with the Soil Seed Bank and the Viable Seed Content of Farmyard Manure and Hay Samples.

Table 6.4 shows the differences in species composition of the New House meadow vegetation, the upper fraction of the soil core, hay samples and manure samples of various ages. Differences between the samples are evident from this table. The comparison of the meadow vegetation with soil seed bank, hay and manure samples from New House Farm, within the PCA framework, gave rise to results similar to those seen at Piper Hole. However, in contrast to the Piper Hole samples, in Figure 6.5a the New House Farm soil seed bank, hay and manure samples form almost one cluster rather than distinctly separate groups. Soil samples giving way to hay samples and then manure samples. The meadow vegetation samples are represented by points mostly in the upper left part of the graph, whilst the soil samples are generally found in the lower right of the graph. Hay and manure samples from New House Farm are generally in the upper right part of the graph.

Table 6.4 The frequency and domin value range of New House vegetation and the mean number of germinating seed and percentage frequency in the upper fraction of the soil core, hay samples and manure samples of various ages.

Species	New House Vegetation		New House Soil Seed Bank		New House Hay		New House Manure	
	Percentage frequency	Domin Value range.	Mean seed	Percentage frequency	Mean seed	Percentage frequency	Mean seed	Percentage frequency
Anthoxanthum odoratum	100	3, 6	58.3	2.1	12.0	100	1.1	37.5
Trifolium spp.	100	4, 7	58.3	1.8	5.1	100		
Bellis perennis	96	4, 7	91.7	49.8	94.2	100	0.6	2 5
Rumex acetosa	92	1, 8			1.1	80	0.3	12.5
Bromus hordeaceus	88	1, 7	41.7	3.1	23.5	80		
Plantago lanceolata	84	4, 8	91.7	7.5	92.4	100	0.6	25
Taraxacum officinale agg.	80	4, 7	50.0	1.0				
Rhinanthus minor	80	1, 8						
Cynosurus cristatus	80	1, 4			1.2	60		
Poa trivialis	68	1, 4	100	24.	90.9	100	7.7	75
Ranunculus spp.	64	4, 7	91.7	10.7	1.0	80		
Filipendula ulmaria	52	1, 6						
Alchemilla vulgaris agg.	48	1, 5	33.3	1.4				
Lolium perenne	48	1, 4			31.5	100	0.5	25
Alopecurus pratensis	48	1, 4	33.3	1.2	2.0	100		
Cerastium fontanum	48	1, 4	100.0	13.3	1.0	60		
Myosotis spp.	44	1	100.0	11.1	10.7	80	0.6	25
Holcus lanatus	32	1, 5	50.0	1.4	0.8	40		
Anthriscus sylvestris	32	1, 5						
Festuca rubra	28	1, 4			0.5	40		
Phleum pratense	28	1, 4			0.7	40		
Geranium sylvaticum	28	1, 4						
Sanguisorba officinalis	28	1, 4						
Ranunculus ficaria	24	1, 4						
Conopodium majus	24	1, 4			0.6	40		

Dactylis glomerata	24	1, 2						
Stellaria media	24	1, 2						
Veronica chamaedrys	24	1						
Cirsium helenioides	20	6,7						
Carex nigra	20	1, 6						
Juncus effusus	16	1, 5	66.7	1.3	0.9	20	0.26	12.5
Rumex obtusifolius	12	1, 5						
Geum rivale	12	1, 4						
Deschampsia cespitosa	12	1, 4			1.2	20		
Carex panacea	12	1, 4						
Caltha palustris	8	3						
Heracleum sphondylium	8	1						
Euphrasia nemorosa	8	1						
Centaurea nigra	4	1						
Cardamine pratensis	4	1	33.3	0.5				
Poa annua	4	1						
Festuca pratensis	4	1			0.3	20		
Agrostis capillaris	4	1			0.5	40		
Briza media	4	1						
Juncus bufonius			83.3	1.3	0.3	20	0.64	37.5
Poa pratensis			41.7	1.1	1.4	80		
Veronica serpyllifolia			33.3	1.6				
Cirsium arvense					0.3	20		
Helictotrichon pubescens					0.3	20		
Polygonum bistorta					0.3	20		
Chamerion angustifolium					0.2	20		
Urtica dioica							0.14	12.5

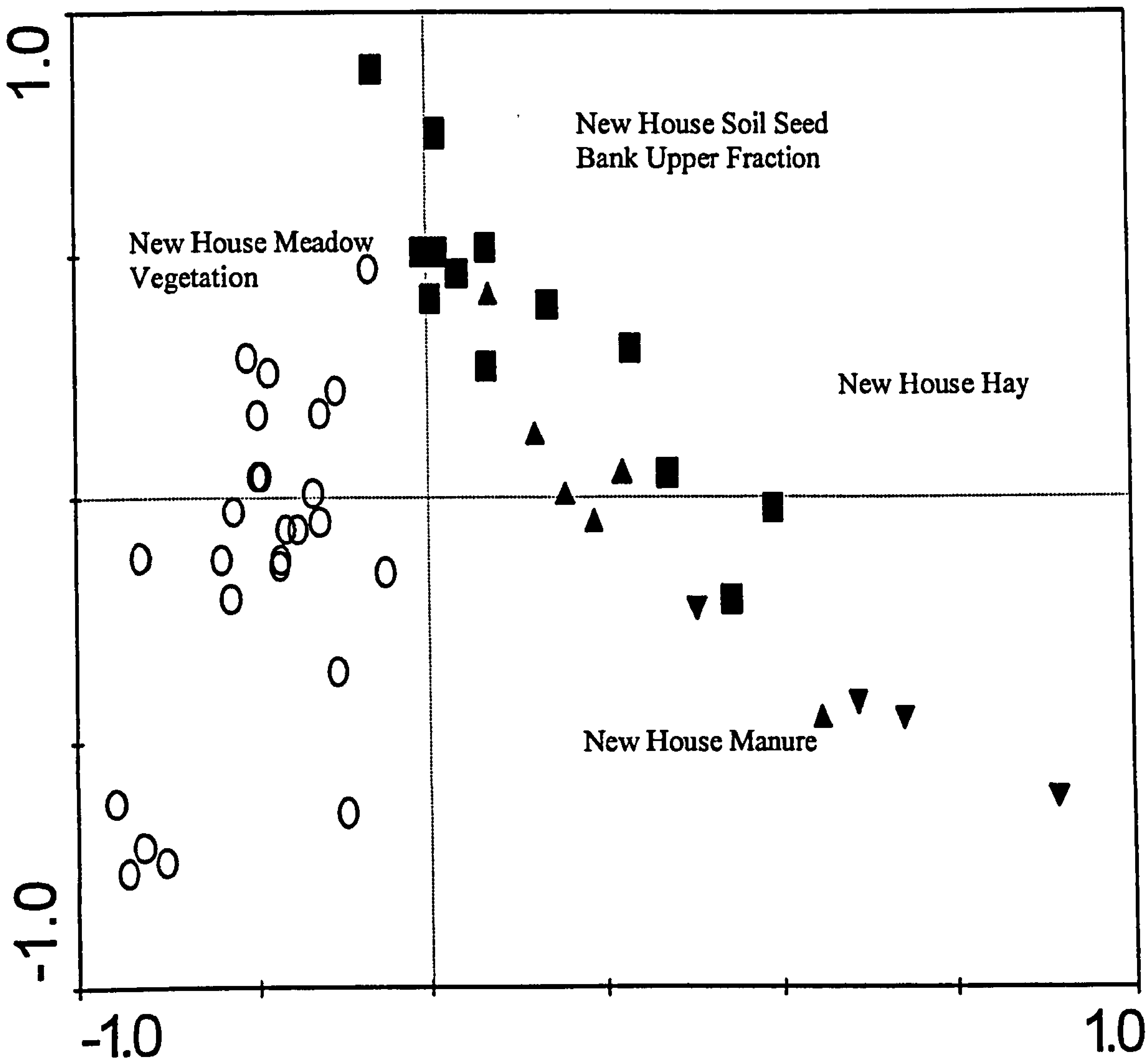


Figure 6.6a PCA plot showing the site ordination of New House Farm ■soil seed bank samples, ▲hay and ▼manure samples and Ο meadow vegetation samples.

Figure 6.6b the species plot shows that the New House hay and manure samples were dominated by *P. trivialis* and *J. bufonius* as was the case with the Piper Hole samples in Figure 6.5b. The early flowering herbs *A. vulgaris* agg. and *B. perennis* along with *Myosotis* spp. and *Cerastium fontanum* are found mostly in the soil samples. Whilst the larger Perennial herbs such as *C. palustris*, *G. sylvaticum* and *F. ulmaria* were only found within the vegetation.

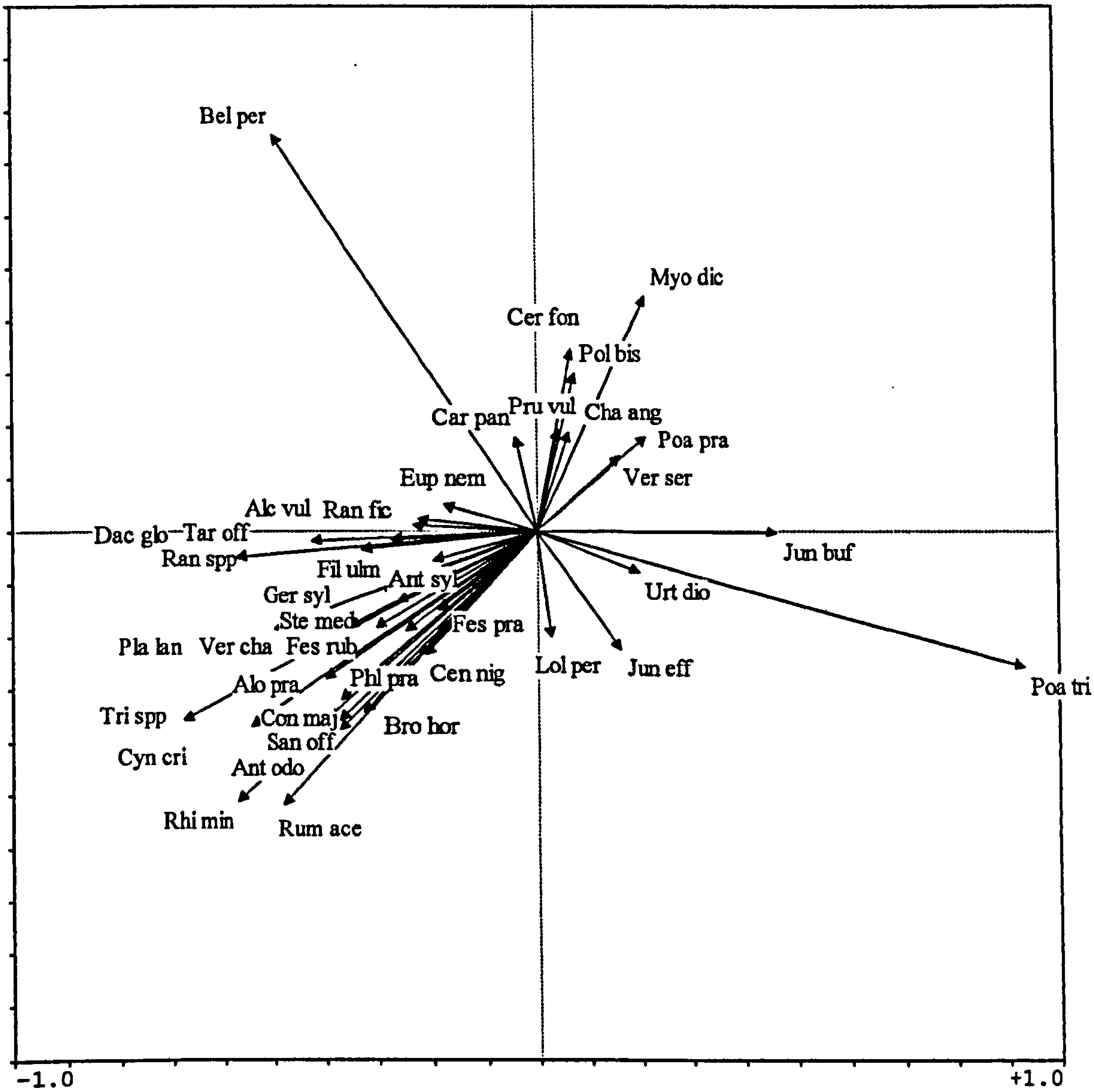


Figure 6.6b PCA plot showing the species ordination of Piper Hole soil seed bank, hay, manure and meadow vegetation.

6.4.5 Comparison of Vegetation, soil seed bank upper fraction, hay and manure from both Piper Hole and New House meadows.

By combining the data sets for the Piper Hole and New House Farm meadow vegetation, upper fraction of the soil seed bank, hay and manure samples it is possible to determine how the two traditionally managed meadow systems compare with each other. Figures 6.7a and 6.7b show the results of the PCA analysis of these data sets.

Figure 6.7a shows that the two farms have distinctly different meadow vegetation. The Piper Hole vegetation is found in the lower left part of the graph whilst the New House farm meadow vegetation is generally found as a separate cluster in the upper left part of the graph. In contrast to the meadow vegetation the manure from the two sites is very similar forming a cluster in the upper right of the graph.

The soil seed bank samples from the two sites are generally clustered separately and are closer to the meadow vegetation from which they originated than the manure samples whilst the hay samples from the two sites are more similar to each other and to the manure samples than the soil seed banks of their respective meadows.

Comparison of Figures 6.7a and 6.7b show the differences in species between the vegetation at New House and Piper Hole Farms. The Piper Hole vegetation is characterised more by species such as *Geranium sylvaticum, G. pratense* and *Sanguisorba officinalis*, whereas the New House Farm vegetation contains more species such as *Rhinanthus minor, Anthoxanthum odoratum, Cirsium helenioides* and *Euphrasia nemorosa*. The soil seed banks from both sites also differ from each other and in general occur as an intermediate between the hay and manure samples and the vegetation. The manure samples from both farms are dominated by *P. trivialis* and *J. bufonius*.

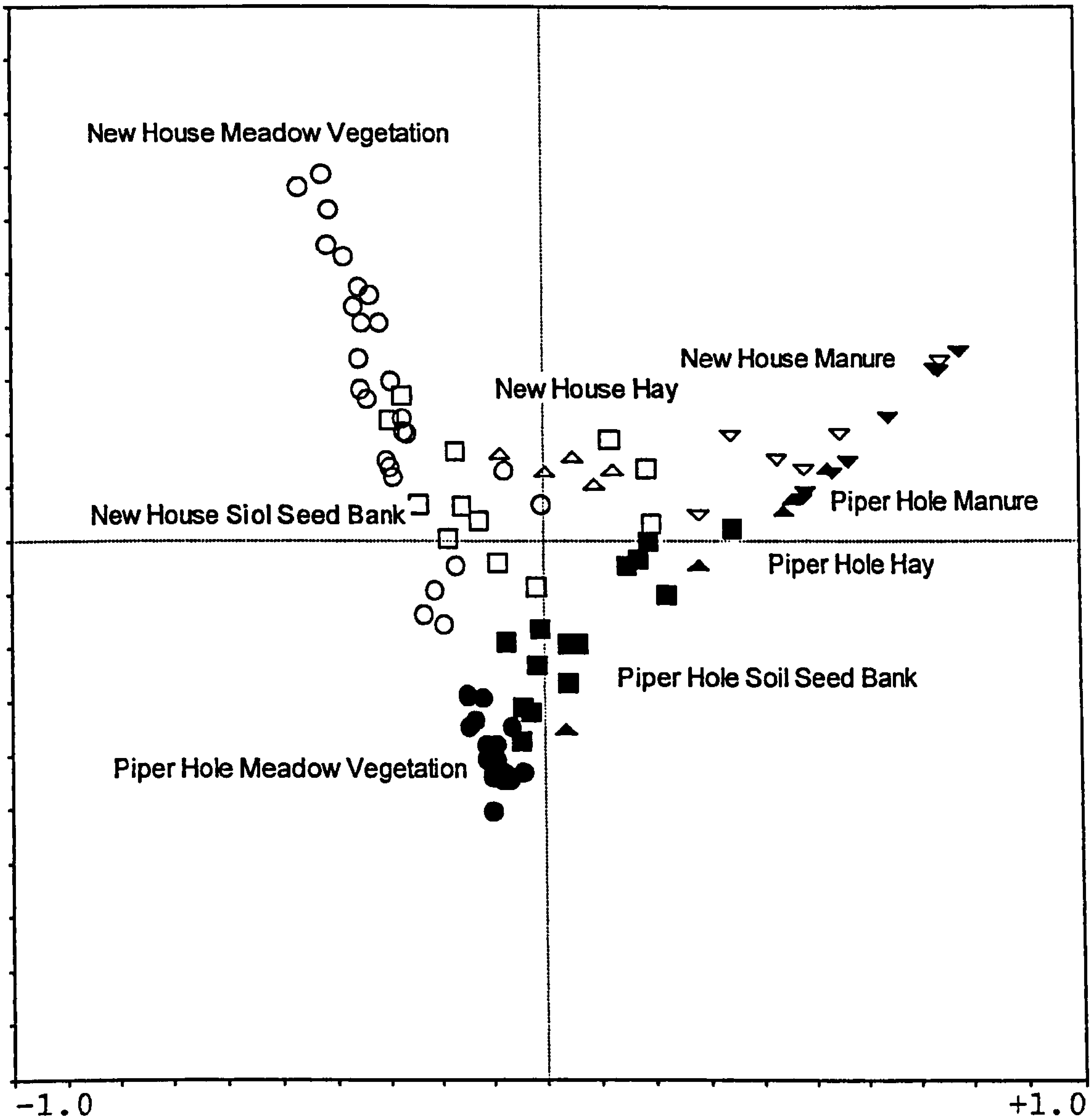
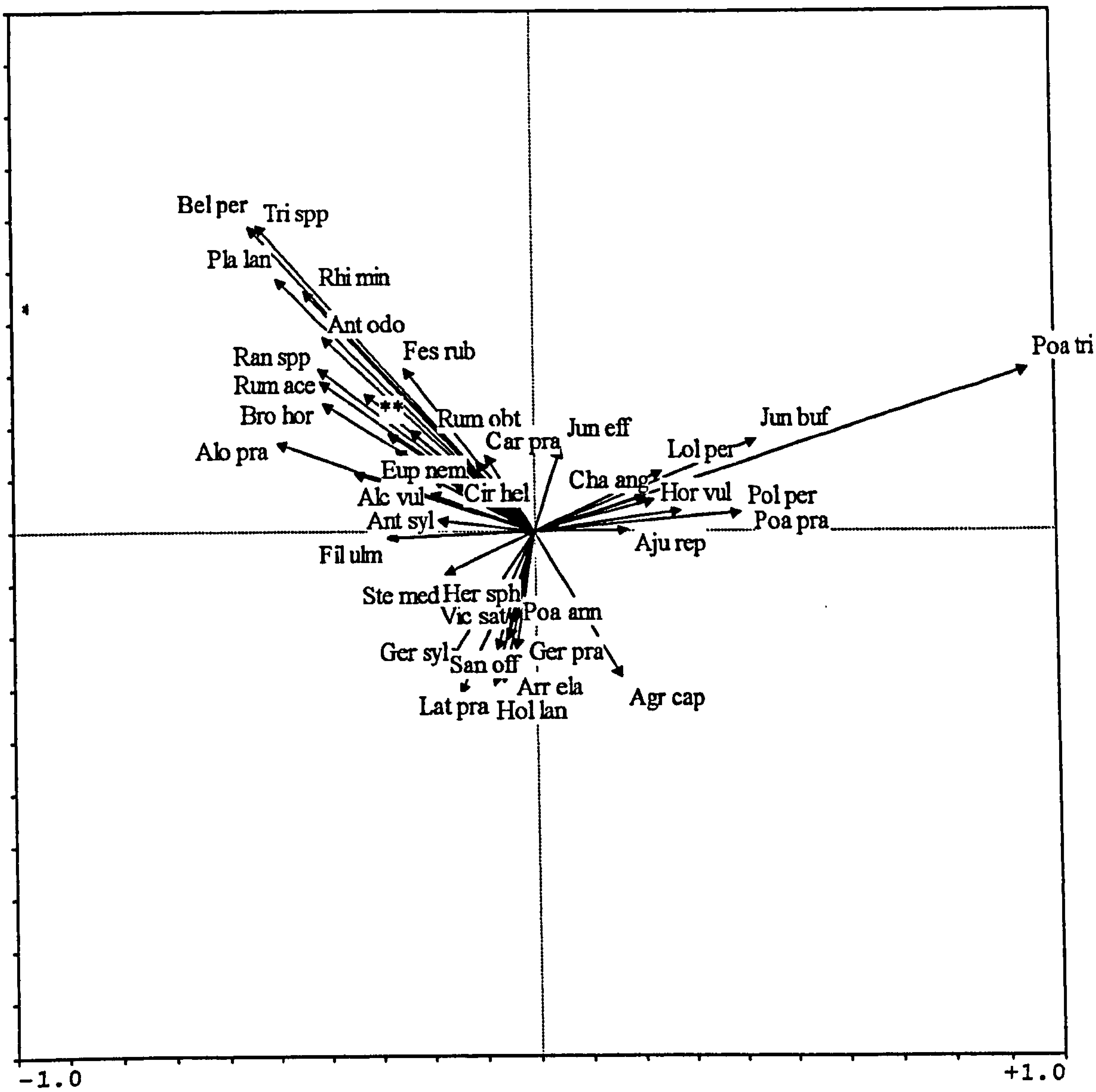


Figure 6.7a PCA plot showing the site ordination of meadow vegetation, soil seed banks, hay and manure from New House Farm and Piper Hole. ○ New House Vegetation, □ New House Seed Bank, △New House Hay ▽ New House Manure, ● Piper Hole Vegetation, ■Piper Hole Seed Bank, ▲ Piper Hole Hay, ▼ Piper Hole Manure.



** Ver cha, Con maj, Tar off and Cyn cri

Figure 6.7b PCA plot showing the species ordination of meadow vegetation, soil seed banks, hay and manure from New House Farm and Piper Hole.

6.4.6 Comparison of NVC sub-communities to the meadow vegetation and viable seed content of hay, manure and soil seed bank at both New House Farm and Piper Hole.

Figure 6.8 shows the distribution of hay, manure and soil seed bank seed contents as well as the meadow vegetation from which it originates at Piper Hole against a framework of various NVC mesotrophic grassland sub-communities. The hay and manure samples are most closely associated with sub-communities of MG7 which are *Lolium perenne* dominated leys and grasslands. In particular the sub-communities are 7D *Lolium perenne-Alopecurus pratensis* grassland,7E *Lolium perenne-Plantago lanceolata* grassland, 7F *Lolium perenne-Poa pratensis* grassland. Some of these samples are also shown as being intermediate to 7E and 6C the *Trisetum flavescens* sub-community of MG6 the *Lolium perenne-Cynosurus cristatus* grassland which is a type of species poor permanent pasture. The 6C sub-community is characterised by an increased cover of *Phleum pratense*.

The seed content of the soil is also shown as being most closely comparable to the species make up of the MG6C sub-community.

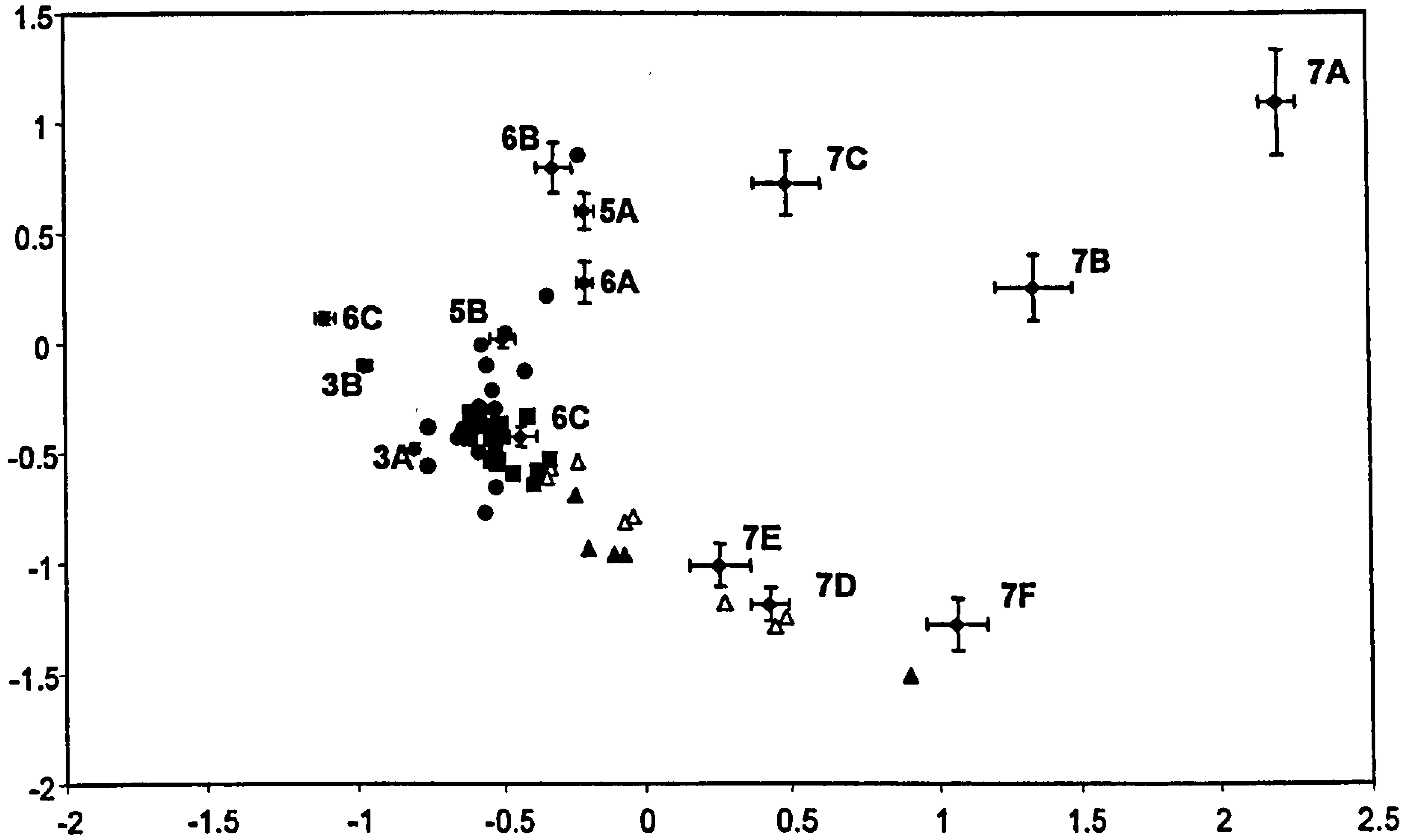


Figure 6.8 PCA plot showing the site ordination of ●NVC sub-communities with standard error bars. Meadow vegetation, soil seed banks, hay and manure from Piper Hole Farm are also included as supplementary data. o Piper Hole Farm Meadow Vegetation, ♦Piper Hole Farm Seed Bank, ■ Piper Hole Farm Hay, ▲ Piper Hole Farm Manure.

The meadow vegetation shows a certain amount of variation with the majority of the samples found nearer to MG3A than either the soil seed bank samples and the hay and manure samples. Some of the hay samples are also found near to the MG5A and 5B which are sub-communities of the *Cynosurus cristatus-Centaurea nigra* grassland typical of grazed hay meadows in the British lowlands in which dicotyledonous species form a significant part of the herbage. A few of the vegetation samples compare to MG6B the more species rich *Anthoxanthum odoratum* sub-community as well as 6A *Lolio-Cynosuretum typicum.*

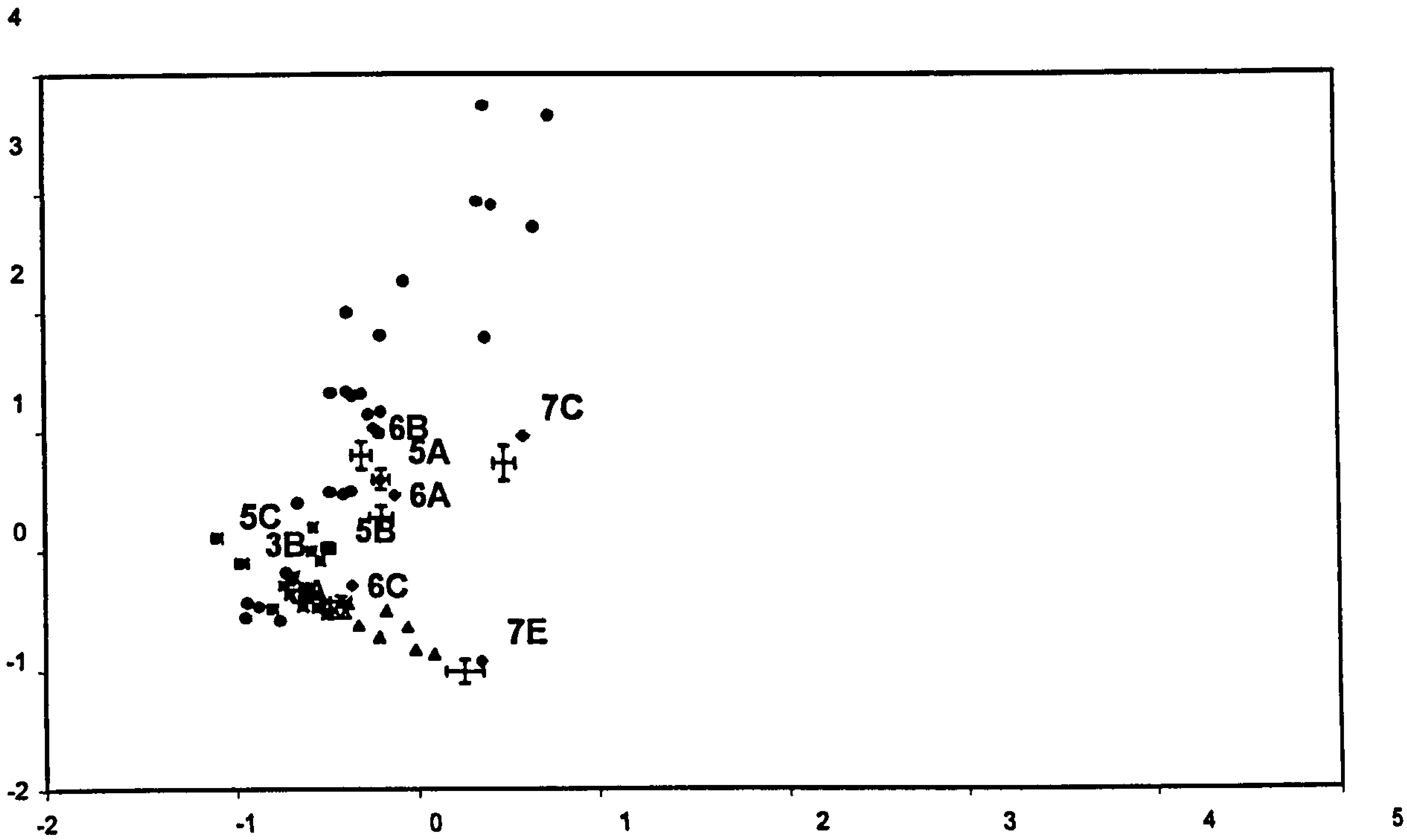


Figure 6.9 PCA plot showing the site ordination of ●NVC sub-communities with standard error bars. Meadow vegetation, soil seed banks, hay and manure from New House Farm are also included as supplementary data. oNew House Farm Meadow Vegetation, ♦New House Farm Seed Bank, ■New House Farm Hay, ▲ New House Farm Manure.

Samples taken from New House Farm when compared to the NVC framework in Figure 6.9 show that the hay and manure samples are found to be comparable to MG6C *Lolium perenne-Cynosurus cristatus* grassland *Trisetum flavescens* sub-community and 7E *Lolium perenne-Plantago lanceolata* grassland whilst the soil seed bank samples are mostly comparable to MG6C as well as MG5B *Cynosurus cristatus-Centaurea nigra* grassland *Galium verum* sub-community. The meadow vegetation samples are much more varied ranging from MG3A the *Bromus hordeaceus* sub community of *Geranium sylvaticum-Anthoxanthum odoratum* grassland through to MG5 sub-communities A and B to MG6 B and others.

It is clear Figures 6.8 and 6.9 that a gap when produced within a meadow at piper Hole is not simply filled by seeds of species germinating from the soil seed bank or from spread manure but that other species of perennial herb such as *G. sylvaticum* for example must be likely to maintain there prominence within the sward through vegetative spread. If all gaps were filled by seed from the seed bank, the meadows would quickly become much less species rich and resemble types of MG6 *Lolium perenne-Cynosurus cristatus* grassland. It should also be noted that using hay from these traditionally managed meadows as an aid to increasing the botanical diversity of poorer swards would lead to little in the way of desirable species being introduced related as it is to MG7 grasslands.

6.4 Discussion

The results presented here support the often reported view that the composition of soil seed banks can differ greatly from the vegetation found above ground (Kirkham and Kent 1997; Akinola *et al.,* 1998; Lopez-Marino *et al.,* 2000). This has also shown to be the case in meadow vegetation (Kirkham and Kent 1997; Jensen 1998; Smith *et al.,* 2001). These results demonstrate that the earlier flowering herbs and grasses within the meadow vegetation are generally found in reasonably high quantities in the soil seed bank, whereas the later flowering perennial herbs are mostly absent from the soil seed bank. This is most likely due to the meadow vegetation being cut prior to seed set in most years (Rodwell and Dring 2001).

Species such as *Poa trivialis* which, in the context of northern hay meadows, flower and set seed in mid season are found within both the soil seed bank and the hay and therefore manure samples. These species could have either become incorporated into the seed bank during the hay cutting and turning process or through the spreading of manure onto the meadows the next spring. At an application rate of 12 tonnes ha^{-1} of 6 month old manure (see Chapter 3) we can estimate that at Piper Hole approximately 138 *P. trivialis* seeds m^{-2} would be spread onto the meadow the extent to which these seeds would either die, germinate or become incorporated into the soil seed bank is unknown. However the upper 5 cm of soil in these meadows contained on average 16 217 seeds m^2 of which 3 917 were *P. trivialis.* This suggests that manure would play a limited role in the seed incorporation into the soil seed bank. There are however a number of earlier flowering species in the seed bank which are not found within the manure. Seed shed prior to the hay cut would account for the abundance of species such as *Rhinanthus minor* and *Alchemilla vulgaris* agg. within the seed bank and species found within the hay are likely to be shed during the hay making process as well as becoming incorporated into the hay (Smith, Pullan and Shiel 1996). The extent to which species such as *P. trivialis* are incorporated directly into the soil seed bank or indirectly via manure spreading would need further experimental work to quantify.

The differences between the soil seed bank and the meadow vegetation highlights the need for input of seed to the meadow system in order to recreate traditional meadow vegetation (Bakker *et al.*, 1996). Without the addition of seed, the reliance upon regeneration from existing soil seed banks for the recreation of hay meadow vegetation would appear to be misguided. The soil seed bank samples show a greater resemblance to relatively species poor plant communities such as MG6 *Lolium perenne-Cynosurus cristatus* grassland, a species poor permanent pasture community in which only dicotyledonous herbs such as *Trifolium repens, Cerastium fontanum, Plantago lanceolata, Ranunculus acris* and *Bellis perennis* are prominent (Rodwell 1992).

The natural rates of plant dispersal into meadows and the incorporation of these species into the vegetation is slow (Hutchings and Booth 1996). The spreading of hay has been suggested as a way of introducing seed into grasslands (Wells, Frost and Bell 1986; Atkinson *at al* 1995; Jones *et al.,* 1995; Mortimer *et al.,* 2002) but as these results show that hay does not contain a large quantity of seeds of the most desirable plants found in Pennine hay meadows. In fact the species make up of the seed content of meadow hay samples was found to be more closely related to MG7 *Lolium perenne* dominated leys and grasslands as well as MG6 *Lolium perenne-Cynosurus cristatus* grassland.

The meadow vegetation at Piper Hole is shown as most closely resembling that of the more species poor *Bromus hordeaceus* sub-community (MG3A) of MG3 and the Vegetation at New House Farm is more variable with some samples resembling MG3A and 5A and 5B and 6A and 6C. It should however be noted that the small quadrat size used in surveying the meadows compared to those on which the NVC data is based would give the meadow vegetation samples a more species poor appearance when compared to the NVC framework than would be the case if larger quadrats were used.

In the context of maintaining diversity within an extant traditionally managed hay meadow it is obvious from the results presented within this chapter that when a gap is produced within the vegetation, colonisation by plants from seed either from the soil seed bank or from within manure are not the only sources of colonisers. Given that the soil samples were taken in the autumn it is possible that some species may have set seed at, or prior to the hay cut, and germinated straight away in the late summer. It is also possible that seed of other species may have dispersed into the meadow from surrounding road side verges and germinated straight away. However given the lack of long distance dispersal capability of many of the species not represented within the soil seed bank or manure (Hodgson *et al.,* 1995), this would appear to be unlikely. It has also been shown that *G. sylvaticum* for example requires a period of cold chilling prior to germination (Hill 2001) and so therefore this species at least, if producing significant amounts of seed would have been expected to be recorded within the soil or hay samples in much greater quantities.

These results as well of those in previous chapters highlight a possible lack of reproduction by seed for a large number of perennial herb species in these meadows; this is one of the reasons that recreation of these meadows once they have been improved is so difficult.

7. The colonisation of gaps produced by small scale disturbances to the sward.

7.1 Introduction

Previous chapters have established the total viable seed content and species composition of farmyard manure, how the seed within farmyard manure is affected by storage and how that manure compares to the seed content and species composition of the soil seed bank. This final experiment involved the investigation of the seed germination in gaps produced when the manure is spread onto the meadow and also whether a species poor sward can be enriched by the application of manure from a traditionally managed species rich system.

Gaps can be produced within hay meadow vegetation by processes such as animal trampling or die back under clumps of manure (the muck spreader does not produce an even coverage of manure). Such gaps could be filled by either vegetative spread of surrounding plants, seeds germinating from the soil seed bank or seed germinating from within the manure. As previous chapters have established the species composition of both the manure and the soil seed bank, it should be possible to use experimentally manipulated plots within the meadow to compare the vegetation that fills the gaps to the species composition of manure, the soil seed bank and the overall species composition of the meadow vegetation.

The mechanisms involved in the replacement of species within vegetation is of great importance in understanding how species richness is maintained (Grubb 1977). Bullock *et al.*, (1994) suggest that the survival and vegetative spread of plants and the establishment of seedlings are key processes in determining the species composition of plant communities. It is clear therefore that the potential seed dispersal and germination niche capacity of manure application should be investigated. The gaps produced when manure is spread could provide niches for the regeneration of species in the meadows which could favour some species over others. For example certain species within acidic grassland in the UK were favoured by regeneration from rabbit dung whilst others were favoured by regeneration from the soil seed bank (Pakeman *et al.*, 1998). In grasslands, patch disturbances provide opportunities for less competitive species to colonise environments of relatively low competitive pressure (Platt 1975; Hobbs and Mooney 1985; Martinsen *et al.*, 1990). The nature of the micro environment within a gap produced by farmyard manure may favour germination of seed from within the manure or from the soil seed bank. Such a micro site would enable competition to be reduced whilst at the same time enabling germination within a nutrient rich environment.

Humphreys *et al.*, (1997) showed that the positive association between cattle slurry application and *Rumex obtusifolius* populations in grassland is probably due more to favoured establishment of seedlings following application of slurry rather than to dispersal of seed within the slurry. Cattle slurry was therefore not only a medium for dispersal but also acted to produce gaps in vegetation that favoured the establishment of seedlings. *R. obtusifolius* populations tend to die out in undisturbed grassland and depend on disturbances to maintain populations. Differences in regeneration niches (Grubb 1977) are thought to help maintain diversity in plant communities so the effect of gaps produced by manure applications could be important for a range of plants within hay meadows.

In a wet semi-natural grassland in Sweden only one species from the seed bank, *Taraxacum officinale*, colonised a gap in the vegetation (Milberg 1993) whilst in acid grassland in England, Pakeman *et al.*, (1998) found that the seedbank accounted for around 40 % of re-colonising seedlings in 0.5 m x 0.5 m gaps. Wind and other non-edozoochorous dispersal accounted for the about the same percentage with the rest being made of seeds germinating from rabbit dung. It is often the nature of the gap that determines the relative importance of seed bank and other sources of colonists (Thompson 2000).

It is widely accepted that a major factor involved in limiting the diversification of species poor swards is a lack of seed dispersal into a site (Bakker, Poschlod and Stryksra 1996; Bakker and Berendse 1999; Smith *et al.*, 2002). A number of authors have reported on the positive effects on species diversity of the application of hay from a species rich site in experiments aimed at restoring meadow vegetation (Wells, Frost and Bell 1986; Atkinson *at al* 1995; Jones *et al.*, 1995; Mortimer *et al.*, 2002), especially when combined with disturbance of the vegetation to provide germination sites for seed (Wells, Frost and Bell 1986). It has previously been assumed that negative introductions to hay meadows sites have been bought in on manure from other farms (Simpson, Hunter and Jefferson 1996). Whilst Bakker *et al.,* (1996) point out the need for the study of "dispersal and germination niches created by dispersal agents". Therefore it is worthwhile investigating what the effects of manure additions from species rich sites to species poor sites may be.

The aim of this experiment was therefore to determine:

1. Whether manure application produces gaps within meadow vegetation.
2. Whether the species colonising any gaps produced were likely to be derived from seed contained within the manure.
3. How vegetation compared when gaps were produced by manure as opposed to by turf removal.
4. If the application of farmyard manure from a traditionally managed source would introduce species to a species poor sward.

7.2 Methods.

7.2.1 Piper Hole.

7.2.1.1 Marking of vegetation blocks.

On the 24/03/00 two blocks of uniform vegetation of 5.0625 m^2 were marked out in the corner of one meadow (Great Bottom) it was agreed with the owner that during the running of this experiment this part of the meadow would not have manure spread upon it. Each plot was further subdivided into 25 cm grid squares, each block comprising of 9 x 9 25 cm squares. The plots were initially marked with canes and string in order to aid the application of the treatments. Following the treatment application metal disks were buried below the surface of the meadow so that the block could be found and marked out again at a later date using a metal detector.

7.2.1.2 Treatment of experimental plots.

Four treatments were then applied at random to 25 cm squares within each block. These four treatments were application of farmyard manure, application of a straw flour/water mix, removal of the vegetation and a no treatment control each treatment was repeated four times. Every treated square had an untreated square surrounding it on all sides.

The plots receiving farmyard manure were treated with manure taken from the midden at Piper Hole Farm. This manure was taken from the same part of the midden that was used to provide manure for the rest of the meadow. Treatment applications were approximately 20 cm diameter, circular and 2 cm deep, and these were placed in the middle of the plots. Whilst this quantity was not weighed, it is fair to assume that this amounted to a higher level of application than the 12 tonne ha^{-1} rate that is spread over the meadows at this farm. However the uneven spread of manure by muck spreaders often gives rise to patches of this approximate size within the meadows, so the treatment was reasonably realistic.

The straw, flour and water mix was used in order to mimic the gap producing qualities of manure whilst not providing any of the seed or nutrients contained in manure. The texture of the mix was manipulated in order to mimic as closely as possible that of the manure used on the other plot. Similar quantities were applied to each plot.

The turf removal plots had the entire $25cm^2$ of turf removed using a trowel to leave a patch of bare ground.

7.2.1.3 Survey of vegetation in plots.

After the application of the treatments, the marker canes were removed and the plots were then left to be treated in the same way as the rest of the meadow until shut up. When grazing was halted for the growth of the hay crop the plots were found again using a metal detector, and the blocks marked out using canes and string.

Once the vegetation was grown to a stage which made identification of the species easier, the species composition and percentage cover of each species within each treatment square were recorded as was the percentage of bare ground. Following the survey the canes and string were removed and the plots left to be treated as the rest of the meadow. It had been intended that this process would be repeated in the summer of 2001 but the outbreak of foot and mouth disease and the subsequent restrictions meant that it was not possible to repeat the survey until the summer of 2002.

7.2.2 Cockle Park Farm.

On 27/03/00 the same process which was carried out at Piper Hole was repeated at Cockle Park Farm, Northumberland (NZ 202 913) on an area of grassland which had been left to regenerate from intensive arable use since 1994. Since 1994 the regenerating grassland has been mown with a flail mower, every two weeks from April to the end of September depending on grass growth. The cut grass clippings were left in situ on all occasions. The sward was very species poor and mostly dominated by grasses such as *Lolium perenne, Holcus lanatus* and *Agrostis* *stolonifera* with *Ranunculus repens* and *Bellis perennis* also common. In this experiment manure from the intensively managed system at this farm was used in addition to manure from Piper Hole, giving an extra treatment. In 2000 and 2001 during June and July the grass was left to grow on and the resulting crop cut and removed. The vegetation was sampled on 27/07/00 and again on 18/07/01.

7.2.3 Statistical Analysis.

7.2.3.1 Piper Hole.

Analysis of variance was carried out using GLM's with Tukey comparisons (95% confidence levels) to determine the proportion of the total cover that was taken up by bare ground in each of the experimental treatments in 2000. Due to the greatly reduced quantity of bare ground in 2002, it was not possible to repeat the analysis for treatments in the second year of sampling. For both years the method was repeated using data on the total number of species analysed.

Analysis of variance was carried out using GLM's with Tukey comparisons (95% confidence levels) to determine effect of treatment on species number for each years data.

Analysis of variance was carried out using General Linear Models (GLM) with Tukey comparisons (95% confidence levels) on the logarithmically transformed total percentage cover of grasses, annual herbs and perennial herbs for each treatment. The analysis was carried out separately for each year of sampling.

The species composition of the meadow vegetation plots in each year and the viable seed content of the soil seed bank (upper 5cm of soil core), hay and farmyard manure were compared using Principal Component Analysis (PCA) with percentage cover data from the vegetation. The PCA ordination was completed using Canoco (version 4) (ter Braak and Smilauer 1998).

7.2.3.2 Cockle Park.

Analysis of variance was carried out using GLM's with Tukey comparisons (95% confidence levels) on the proportion of the total cover that was taken up by bare ground in each of the experimental treatments in 2000. Due to the greatly reduced quantity of bare ground in 2001 it was not possible to repeat the analysis for treatments in the second year of sampling. For both years the method was repeated with data on the total number of species analysed.

Analysis of variance was carried out using GLM's with Tukey comparisons (95% confidence levels) on the effects of treatments on the % cover of the more common individual species where necessary to maintain a normal distribution of residuals the data were square root transformed.

7.3 Results

7.3.1 Piper Hole Plots.

7.3.1.1 The extent of bare ground produced in meadow vegetation by each treatment.

Table 7.1 shows the percentage of bare ground in each year of recording for each treatment. It is clear that in all but the control areas a quantity of bare ground had been produced by the treatments and that by the second recording this bare ground had been filled by vegetation. A list of the species composition with percentage cover values for each species and the extent of bare ground within each plot is given in Appendix 12.

Table 7.1 The effect of treatment on the mean (n=8) percentage ±SE of bare ground recorded following each treatment in both years of recording with Results of Tukey comparisons where applicable.

Treatment	Mean (±SE) Extent of bare ground (%)	
	2000 (±3.21)	2002
Turf removal	46.75^{a}	0 (0)
Control	0^{b}	2.5 (1.63)
FYM	23.13^{bc}	0 (0)
Simulated FYM	13.75^{bc}	1.87(1.31)

In the year 2000 there was a significant ($F_{3,27}$ = 34.39, P<0.001) difference in the amount of bare ground between treatments. The physical removal of all the vegetation in the plots in the spring resulted in a mean of 46.75% bare ground when the recording was carried out in the same summer. This was significantly higher than in all the other treatments. The application of farmyard manure and simulated farmyard manure produced significantly more bare ground than was recorded in the control plots. No significant difference was recorded between the two blocks of vegetation ($F_{3,27} = 3.91$, $p>0.05$).

Due to the almost complete absence of bare ground recorded in 2002 it was not possible to carry out statistical analysis for this parameter.

7.3.1.2 The effect of treatments on the number of species recorded in meadow vegetation by each treatment.

The number of species in each plot is shown in Table 7.2. In 2000 there was a significant ($F_{3,27} = 3.48$, $P=0.029$) difference in the number of species between the treatments. However, whilst the Anova indicated significant differences the results of the Tukey comparison failed to show any significant differences. From Table 2, the suggestion is however that the turf removal treatment contained less species in 2000 than the other treatments. No significant differences ($F_{3,27} = 1.14$, $P=0.294$) were shown between the two blocks of vegetation.

Table 7.2 The effect of treatment on the mean (n=8) ±SE number of species in each plot.

Treatment	Mean (± SE) number of species in each plot	
	2000(±0.53)	2002 (±0.78)
Turf removal	10.63	10.63
Control	13.0	12.4
FYM	13.13	11.63
Simulated FYM	11.0	11.5

The second set of recordings made in 2002 gave rise to no significant differences between either treatments ($F_{3,27} = 1.01$, $P=0.404$) or blocks ($F_{3,27} = 1.14$, $P=0.294$).

7.3.1.3 The cover of annual herbs, perennial herbs and grasses in each treatment.

In 2000 due to the much greater cover of vegetation in the control plots, these had significantly higher % cover of annual herbs ($F_{3,28}$=21.94, P<0.001), perennial herbs ($F_{3,28}$=10.25, P<0.001) and grasses ($F_{3,28}$=21.94, P<0.001) than plots which received the other treatments. The mean values are shown in Table 7.3. Conversely the plots which had the turf removed creating bare ground contained relatively lower values of cover for the plant types.

Table 7.3 The mean (n=8) ±SE % cover total of all annual herbs, perennial herbs and grasses from each treatment in each year showing the results of Tukey tests (95% confidence level) for the comparison of treatment effects on the % cover of each group of plants.

	Mean (±SE) percentage cover					
	2000			2002		
Treatment	Annual Herb (±1.79)	Perennial Herb (±3.16)	Grass (±2.59)	Annual Herb (±4.21)	Perennial Herb (±8.99)	Grass (±4.76)
Turf removal	4.88	8.75	7.63	16.25	43.5	34.63
Control	27.88[a]	39.63[a]	18.5[a]	27.38	50.13	22.88
FYM	8.25	13.63	9.00	22.63	61.5	22.00
Substitute FYM	10.13	16.5	9.5	24.13	60.75	23.13

Following the re-growth of the vegetation by 2002 there were no statistically significant differences between treatments on the percentage cover of annual herbs ($F_{3,28}$=1.14, P=0.350), perennial herbs ($F_{3,28}$=1.07, P=0.377) or grasses ($F_{3,28}$=0.58, P=0.632). However, values for the percentage cover of annual and perennial herbs are generally lower following turf removal whilst the grass values are higher than was seen in the other treatments.

Results of Anova with Tukey test comparisons showed statistically significant differences between types of species for controls ($F_{2,21}$=9.00, P=0.002) in 2000. Annual and perennial herbs were significantly higher in cover than grasses. In the turf removal plots there were also significant differences ($F_{2,21}$=4.41, P=0.025). Perennial herbs and grasses were recorded as having higher cover than the annual herbs.

In contrast in the first year of sampling the farmyard manure ($F_{2,21}$=1.17, P=0.331) and simulated farmyard manure ($F_{2,21}$=1.61, P=0.224) plots showed no significant differences between the cover of annual herbs, perennial herbs and grasses.

The sampling in 2002 showed significant differences ($F_{2,21}$=4.28, P=0.028) between type of species for the control plots. Tukey test (95% confidence level) showed that perennial herbs were found with a higher % cover than grasses and annual herbs.

The turf removal plots by 2002 gave rise to significant ($F_{2,21}$=4.91, P=0.018) differences between type of species. Perennial herbs were found with higher cover than grasses and annual herbs. The same pattern was seen with farmyard manure plots ($F_{2,21}$=9.62, P=0.001) and simulated farmyard manure plots ($F_{2,21}$=10.01, P=0.001). In all treatments in 2002 perennial herbs had significantly higher cover than annual herbs and grasses.

7.3.2 Comparison of vegetation in 2000 and 2002 with soil seed bank and hay and manure samples.

7.3.2.1 Control Plots.

The control plots in both 2000 and 2002 on Figure 7.1a are separate from the soil seed bank and hay and manure clusters. The control plot vegetation points are generally clustered in the upper left section of the diagram with most of the variation between the samples displayed along the y-axis. The control plots differ from each other between the years of sampling. This is most likely due to the normal dynamics of the diverse sward given the 2 year gap between sampling. The soil seed bank samples are found below the control plot samples with some of these samples intermediate to the hay and manure samples which are with one exception found to the right of the vegetation plots and soil seed bank samples.

Comparison of the species plot (Figure 7.1b) with the samples plot (Figure 7.1a) enables the identification of those species which are most representative of the separate clusters. The control plots are therefore characterised by *Trifolium pratense, Rumex acetosa* and *Anthriscus sylvestris* and to a lesser extent by *Geranium sylvaticum, Filipendula ulmaria* and *Sanguisorba officinalis*. In contrast the soil seed bank samples are more associated with early flowering perennial herbs such as *Bellis perennis* and *Alchemilla vulgaris* agg., annual herbs such as *Rhinanthus minor* and *Cerastium fontanum* as well as grasses such as *Holcus lanatus* and *Anthoxanthum odoratum*. The hay and manure samples are dominated by *Poa trivialis* and other grasses with the rushes *Juncus effusus* and *Juncus bufonius* prominent.

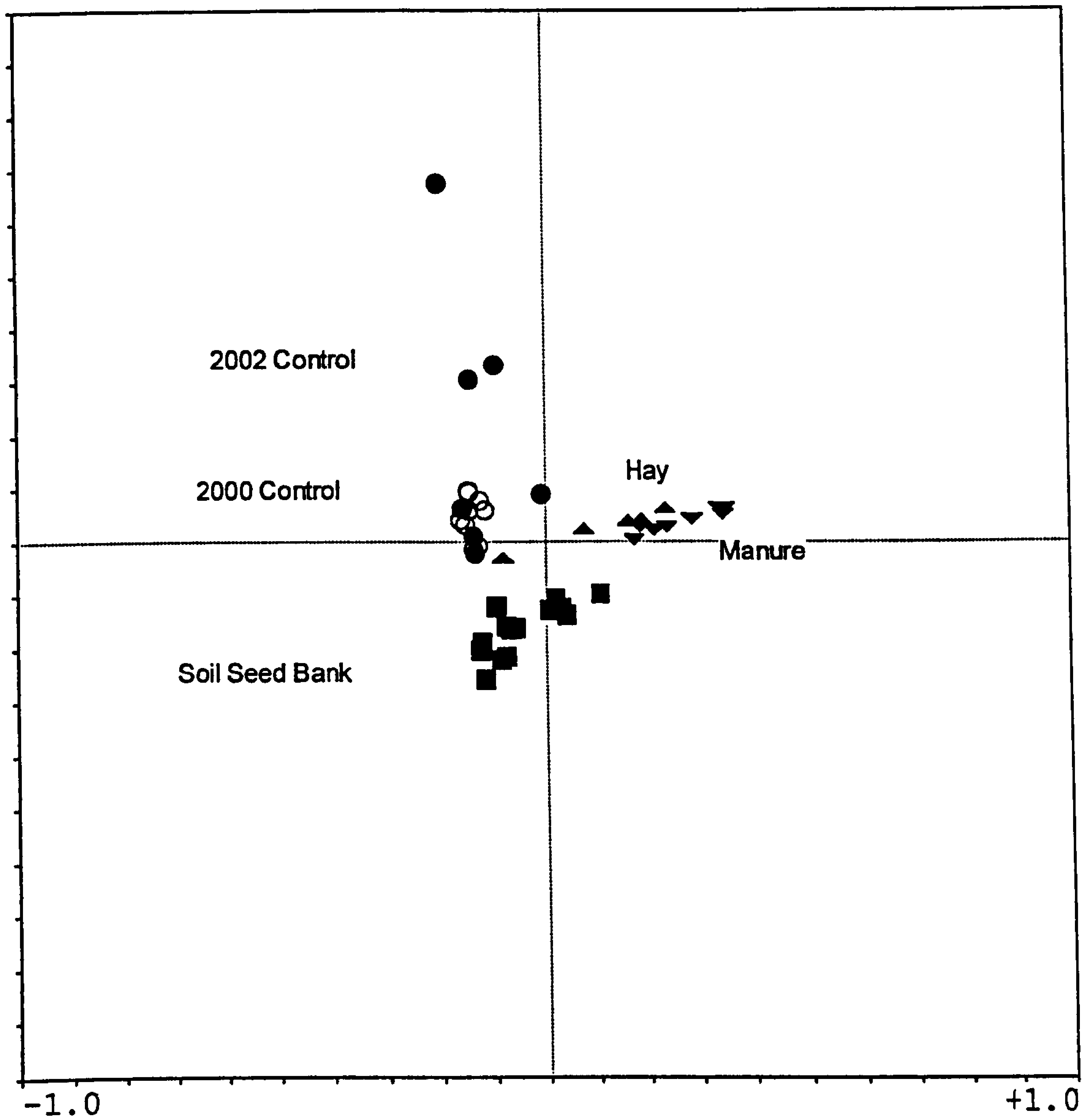
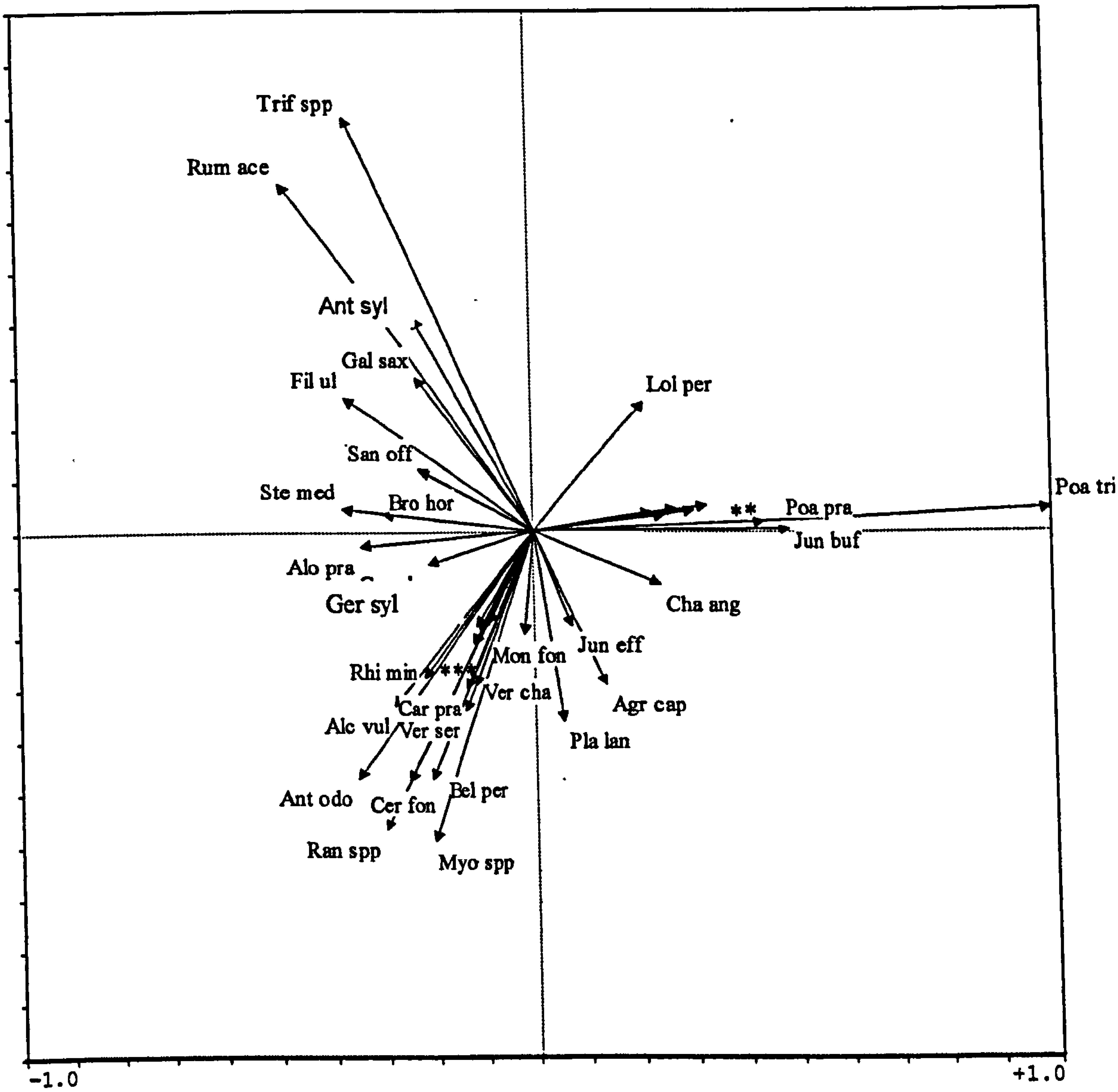


Figure 7.1a. PCA plot showing the site ordination of the control plots in 2000 ●and 2002 ○, compared to soil seed bank■ (upper fraction) samples as well as hay ▲and manure▼ samples.



** Des ces, Poa ann, Hor vul, Pol per and Fes pra
*** Cir arv, Rum obt and Cyn cri

Figure 7.1b The PCA plot showing the species ordination of the control plots in 2000 and 2000 as well as the soil seed bank and hay and manure samples.

7.3.2.2 Turf removal plots.

Figure 7.2a is the samples plot showing the vegetation in the bare ground plots in both years as well as the soil seed bank and hay and manure samples. The soil seed bank samples are shown as being distinctly separate groups from the vegetation in the plots from either year of sampling. The hay and manure samples are also shown as forming a distinctly separate group on the left of the plot.

The points marking out the vegetation in the bare ground plots in 2000 are found in a cluster along the x axis. The same plots sampled in 2002 are in general found higher up in the diagram demonstrating a change in the species composition of the plots, between the two sample dates. The samples from 2002 also appear to be more spread out indicating an increased heterogeneity between the samples.

Figure 7.2b the species plot shows which species give rise to the differences between these separate clusters. *Poa trivialis* dominated the manure samples whilst the soil seed bank samples are characterised by *Myosotis* spp, *Ranunculus* spp. The vegetation in the bare ground plots in 2002 showed an inconsistent pattern in differences from the vegetation in the same plots in 2000. The changes seem to mainly be associated with increased cover of *Trifolium pratense, Rumex acetosa* and *Anthriscus sylvestris.* However some of the 2002 plots are more characterised by species such as *Filipendula ulmaria* and *Sanguisorba officinalis.*

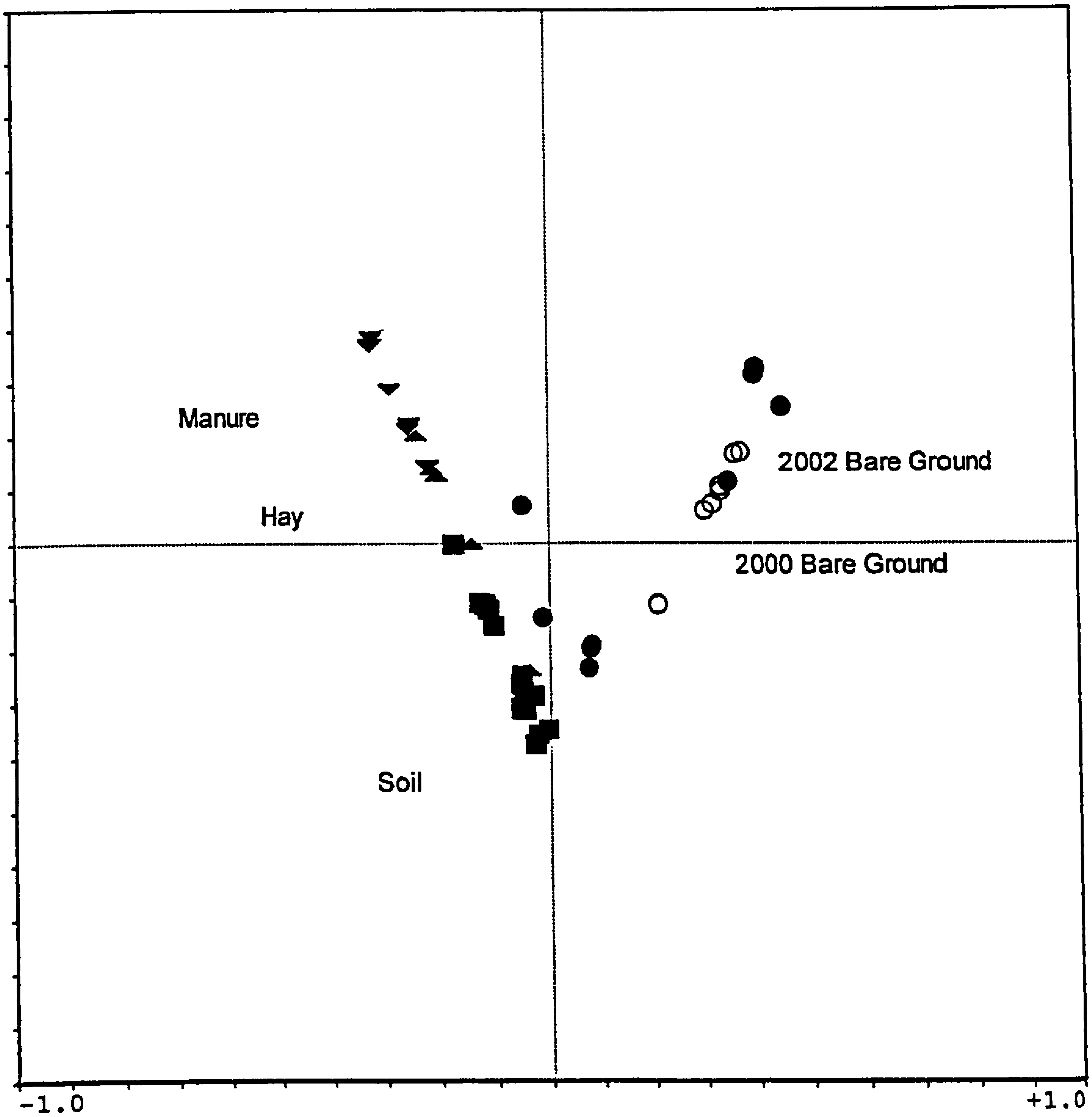
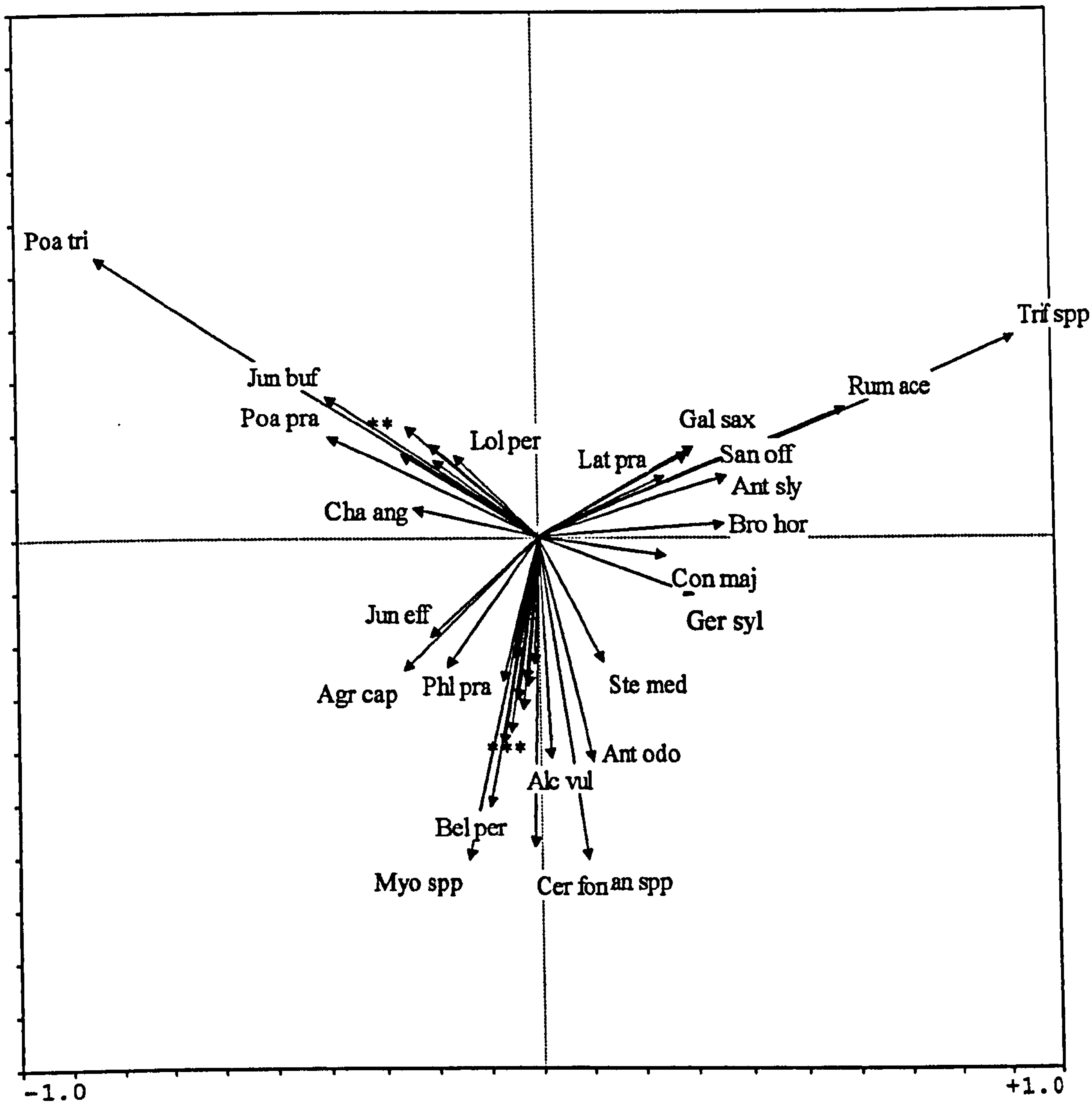


Figure 7.2a. PCA bi-plot showing the site ordination of bare ground plots in 2000○ and 2002 ●, compared to soil seed bank■ (upper fraction) samples as well as hay ▲and manure▼ samples.



** Des ces, Poa ann, Hor vul, Pol per and Fes pra.

*** Rhi min, Ver ser, Ver cha, Car pra, Cyn cris, Alo pra, Urt dio, Hol lan, Mon fon and Rum obt.

Figure 7.2b The PCA plot showing the species ordination of bare ground plots in 2000 and 2000 as well as the soil seed bank and hay and manure samples.

7.3.2.3 Manure Plots.

Figure 7.3a the samples plot of the farmyard manure plots in both years as well as hay and manure samples and the soil seed bank show distinctly different groups. The soil seed bank samples form one grouping as do the hay and manure samples. In general the 2002 farmyard manure plots are represented by points above the x axis whereas the 2000 plots occur below the x axis.

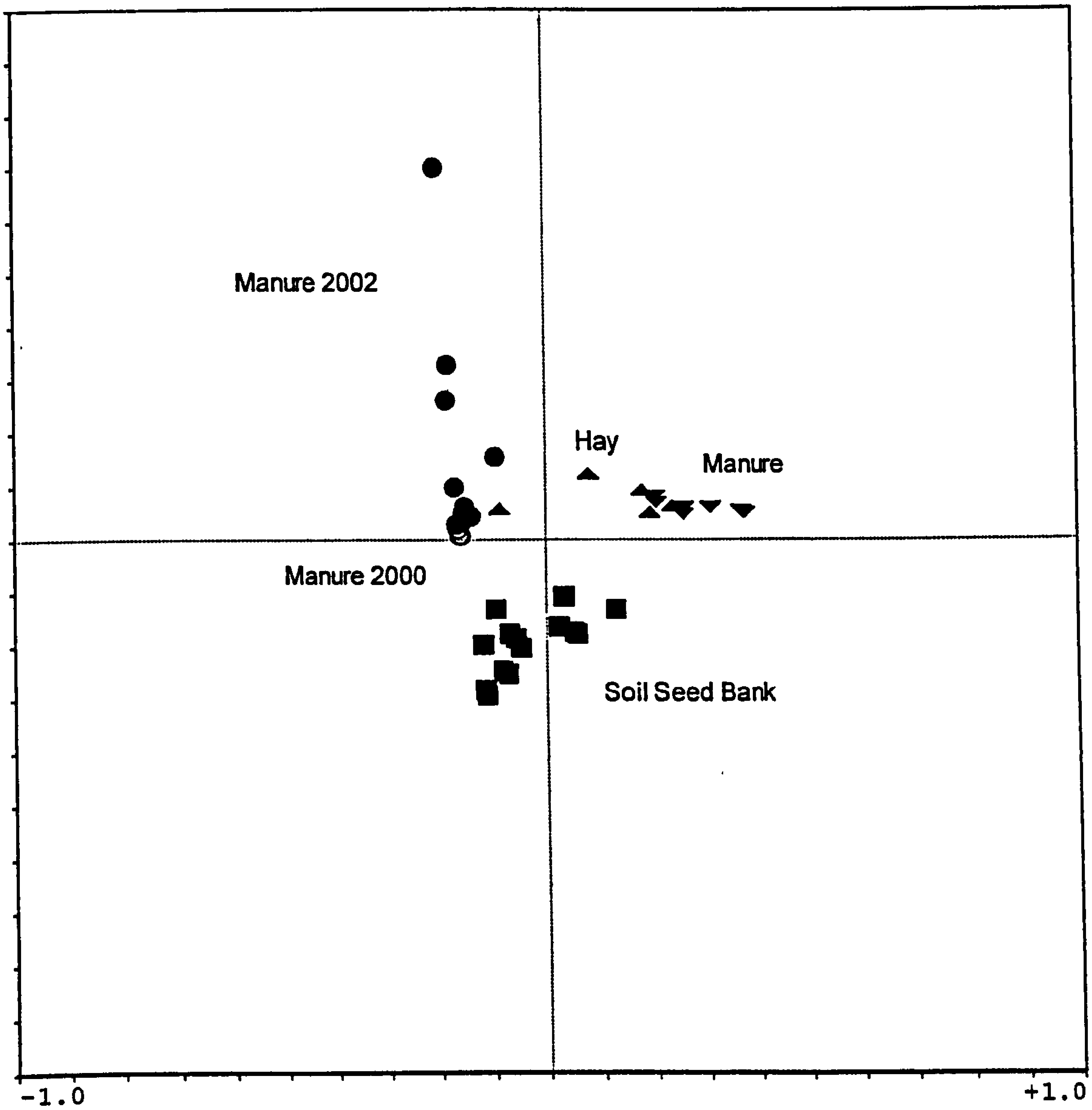
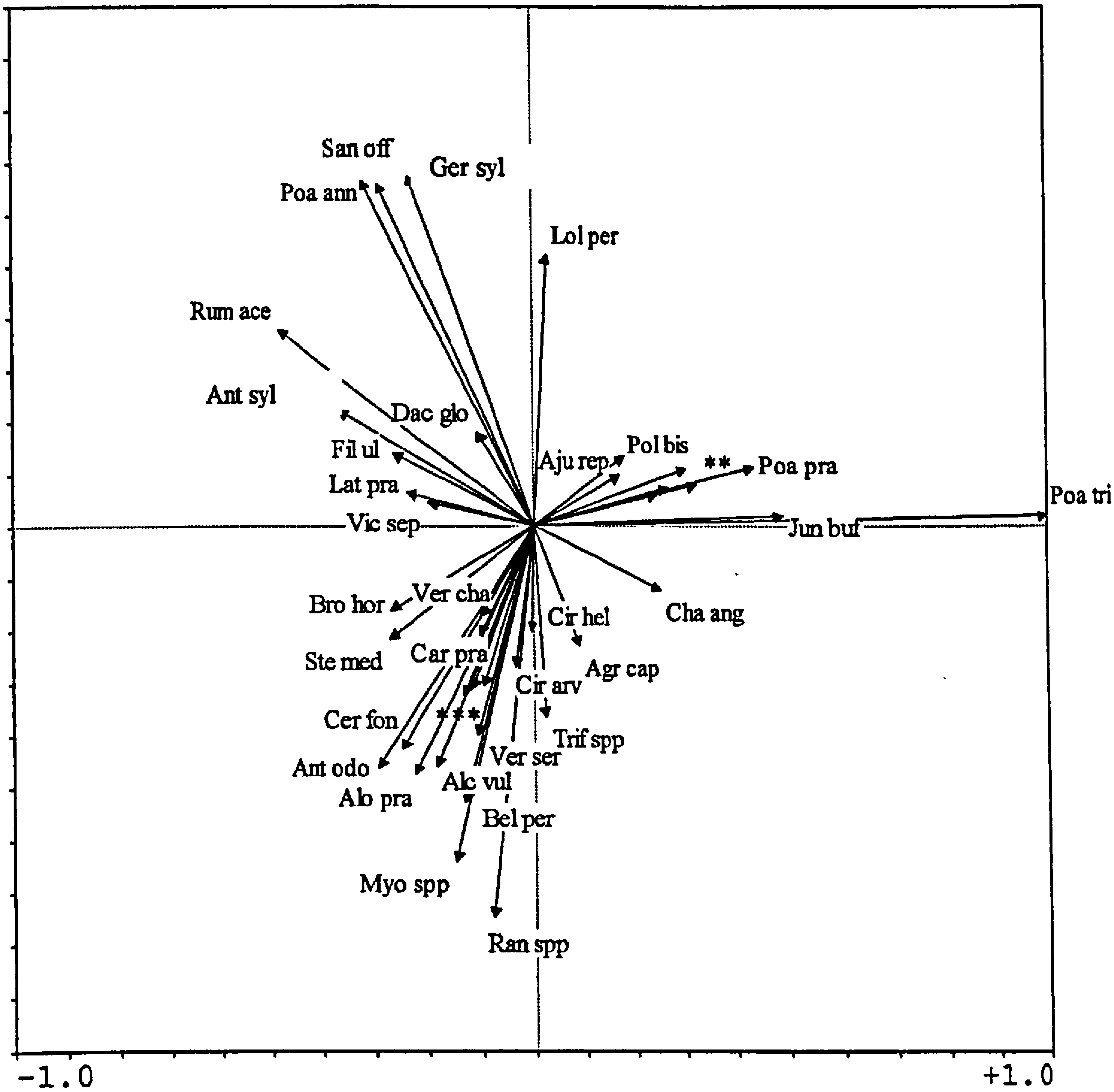


Figure 7.3a. PCA plot showing the site ordination of farmyard manure plots in 2000 ●and 2002 ○, compared to soil seed bank■ (upper fraction) samples as well as hay ▲and manure▼ samples



** Pol per,Fes pra, Hor vul and Des ces.

*** Mon fon, Rum obt and Rhi min

Figure 7.3b The PCA plot showing the species ordination of farmyard manure plots in 2000 and 2002 as well as the soil seed bank and hay and manure samples.

From the species plot (Figure 7.3b) it is clear that by 2002 the plots which received farmyard manure were characterised more by *Geranium sylvaticum, Sanguisorba officinalis* and *Filipendula ulmaria* as well as species such as *Rumex acetosa* and *Anthriscus sylvestris* than the same plots in 2000. These species contrast to those found within the farmyard manure samples which are dominated by *Poa trivialis* or the soil seed bank samples which contain a range of herbs such as *Ranunculus sp,* *Myosotis sp. Bellis perennis* and grasses such *as Anthoxanthum odoratum* and *Alopecurus pratense.*

7.3.2.4 Manure substitute plots.

The samples plot of the farmyard manure substitute plots as well as the soil seed bank and hay and manure samples (Figure 7.4a) show the hay and manure as a separate group as are the soil seed bank samples. The 2002 samples from the plots which received farmyard manure substitute are split, some appearing above the x axis whilst others appear below it. The same plots in 2000 all appear below or in one case just slightly above the x axis. Figure 7.3b shows the 2002 plots above the x axis are characterised by plants such as *Geranium sylvaticum* and *Sanguisorba officinalis* as well as *Alchemilla vulgaris* and *Rumex obtusifolius*. Whilst the plots in 2000 and those 2002 plots found below the x axis are characterised by *Trifolium pratense, Rumex acetosa* and *Filipendula ulmaria.*

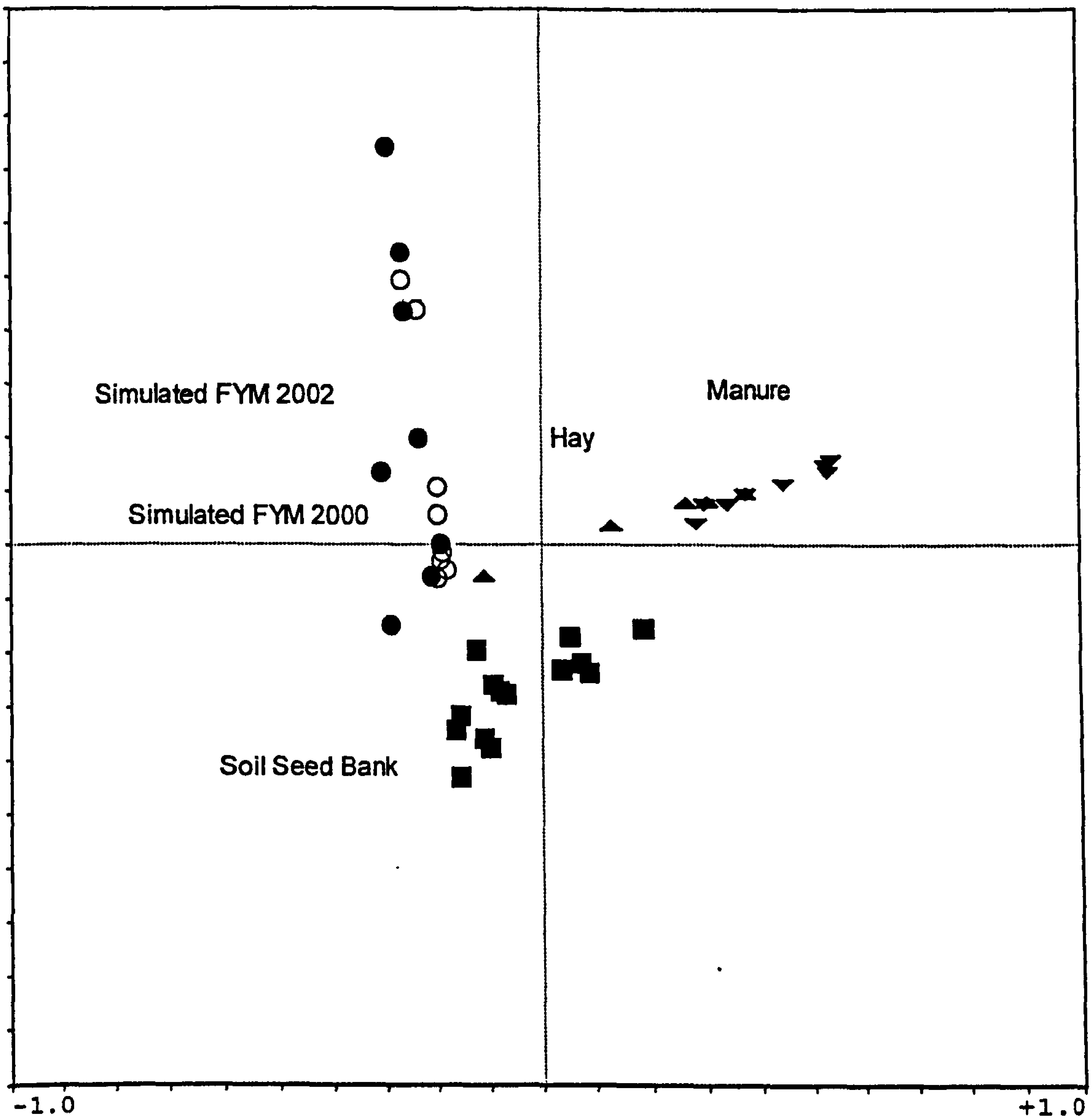
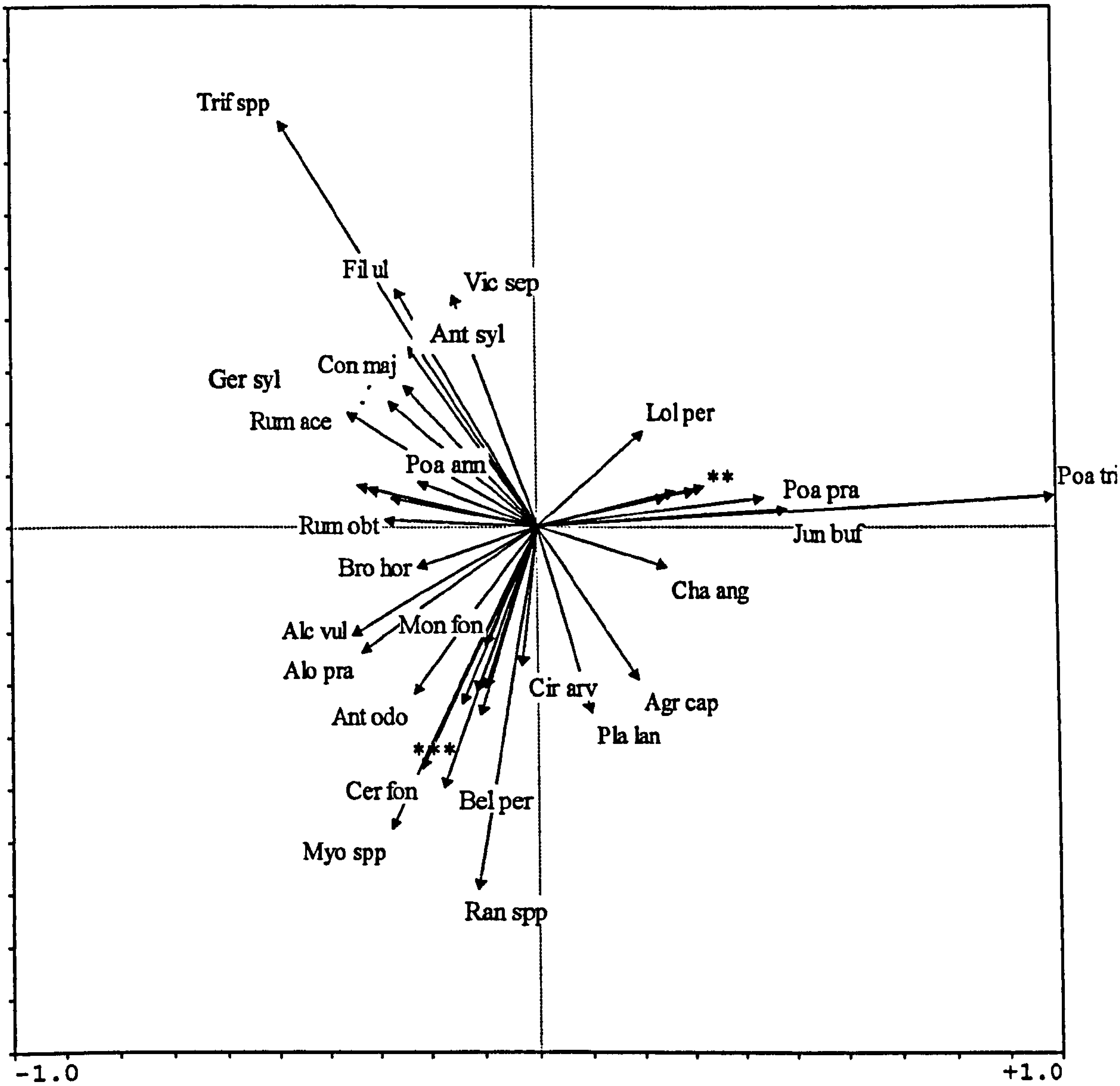


Figure 7.4a. PCA plot showing the site ordination of farmyard manure substitute plots in 2000 ●and 2002 ○, compared to soil seed bank■ (upper fraction) samples as well as hay ▲and manure▼ samples.



** Pol per,Fes pra, Hor vul and Des ces.

***Ver cha, Ver ser, Rhi min and Car pra.

Figure 7.3b The PCA plot showing the species ordination of farmyard manure substitute plots in 2000 and 2000 as well as the soil seed bank and hay and manure samples.

7.3.3 Cockle Park Plots.

7.3.3.1 The extent of bare ground produced by each treatment.

Within the two experimental blocks set up at Cockle Park Farm it is clear that the application of farmyard manure of both types in the spring of 2000 gave rise to greatly increased quantities of bare ground in the summer of 2000. This is shown in Table 7.4, which also shows that the quantity of bare ground in those plots which had the vegetation removed was higher than the two types of manure application and the manure substitute. However even the manure substitute plots contained more bare ground than the control plots. This relationship was shown to be statistically significant $F_{4,44}=121.49$, $P<0.001$.

Table 7.4 The effect of treatment on the mean (n=10) percentage ±SE of bare ground recorded following each treatment in both years of recording. Results of Tukey's comparisons shown for 2000.

Treatment	Mean (±SE) Extent of bare ground (%)	
	2000(±7.79)	2001
Vegetation removal	89.3^{a}	6.0 (3.40)
Control	2.1^{b}	0.0(0.0)
Piper Hole FYM	$71.2^{b,c}$	0.0(0.0)
Cockle Park FYM	$62.0^{b,c}$	1.0(1.0)
FYM substitute	$41.0^{b,c}$	6.5(3.66)

In 2001 the levels of bare ground were greatly reduced in all of the treatment plots. The reduction in the quantity of bare ground was such that insufficient data were available to carry out any statistical analysis.

7.3.3.2 The effect of treatment on the number of species recorded.

Table 7.5 shows that there were no significant differences in the number of species between experimental treatments in either 2000, $F_{4,44}$=2.37, P=0.067, or 2001, $F_{4,44}$=1.13, P=0.353.

Table 7.5 The effect of treatment on the mean (n=10) ±SE number of species in each plot.

Treatment	Mean (±SE) number of species in each plot	
	2000 (±0.36)	2001 (±0.39)
Vegetation removal	3.6	5.8
Control	4.6	5.3
Piper Hole FYM	3.9	5.2
Cockle Park FYM	4.7	6.0
FYM substitute	4.9	5.2

Table 7.6 shows the total number of species within all the plots of each treatment. The total number of species found within the two blocks of vegetation was 15 in 2000 and 16 in 2001 the new species introduced was *Rumex acetosa* a very small quantity of which was recorded within one of the plots which received Cockle Park manure.

Table 7.6 The total number of species recorded within plots of each treatment in both 2000 and 2001.

Treatment	Total number of species in plots of each treatment (n=10)	
	2000	2001
Vegetation removal	10	12
Control	11	11
Piper Hole FYM	8	13
Cockle Park FYM	12	14
FYM substitute	14	11
Total	15	16

7.3.3.3 The species composition of Cockle Park plots in 2001.

The most common species in the experimental plots in 2001 did not differ to any great extent following the application of the treatments in 2000. There is a suggestion that turf removal caused a reduction in cover of *Trifolium repens* and an increase in cover of Holcus *lanatus*. Neither of these differences were found to be significant however ($F_{4,44}$=0.92, P=0.495 and $F_{4,44}$=0.98, P=0.429 respectively).

None of the other differences in species cover were found to be significant of these more common species and the lack of data made statistical analysis of the other species impossible.

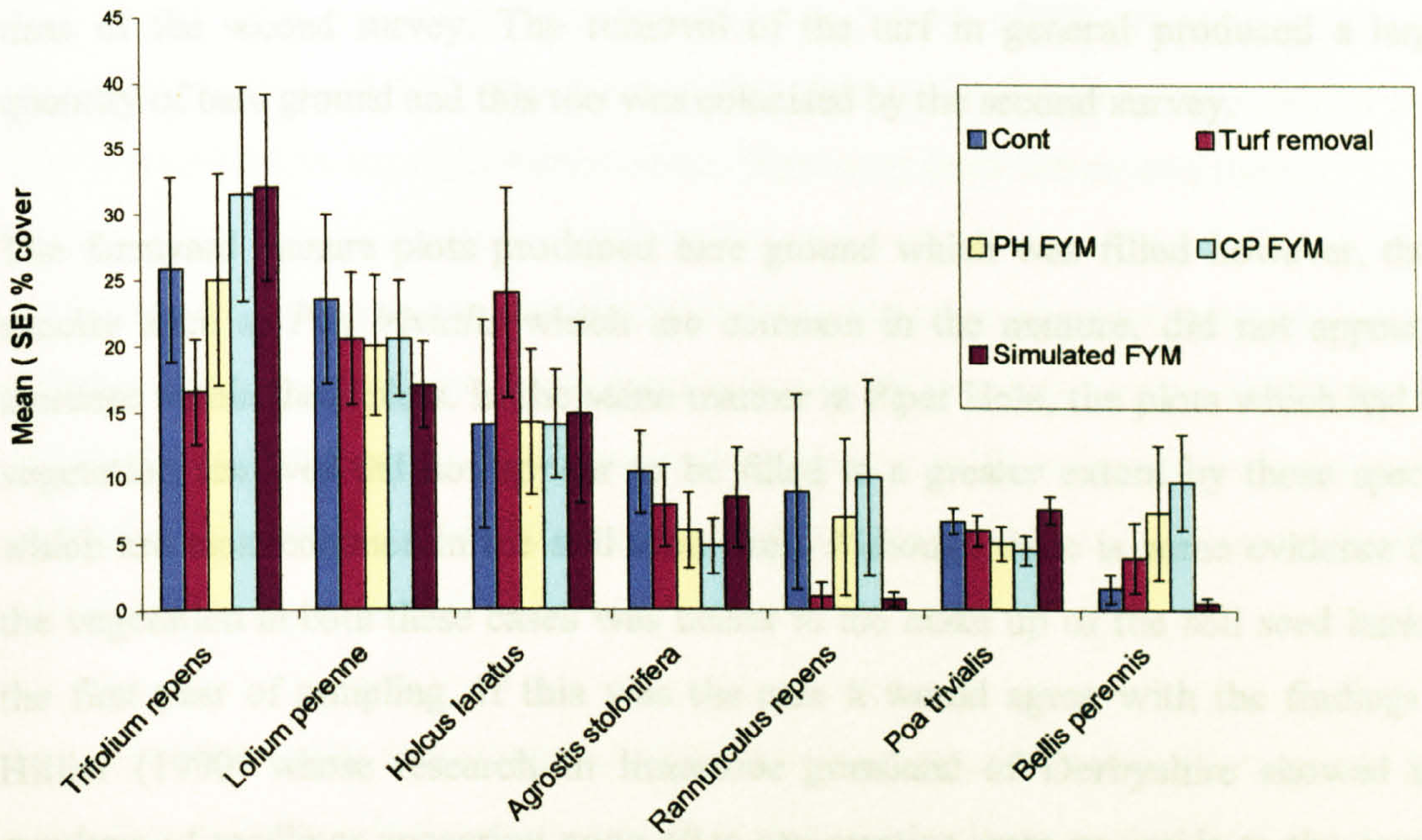


Figure 7.4 The mean (±SE) percentage cover of some of the main species found in the Cockle Park plots of each treatment.

7.4 Discussion

In both experiments the farmyard manure and manure substitute produced a significant quantity of bare ground which was then filled with colonising plants by the time of the second survey. The removal of the turf in general produced a larger quantity of bare ground and this too was colonised by the second survey.

The farmyard manure plots produced bare ground which was filled however, those species such as *Poa trivialis* which are common in the manure, did not appear to increase within these plots. In the same manner at Piper Hole, the plots which had the vegetation removed did not appear to be filled to a greater extent by those species which are most common in the soil seed bank. Although there is some evidence that the vegetation in both these cases was nearer to the make up of the soil seed bank in the first year of sampling. If this was the case it would agree with the findings of Hillier (1990) whose research in limestone grassland of Derbyshire showed that numbers of seedlings appearing soon after gap creation were no guide to the species surviving two years later.

At Piper Hole the plots treated by the removal of the vegetation when compared to the control plots contained a higher cover of the grass *Anthoxanthum odoratum* which in the ordination of the control plots was found to be more common within the soil seed bank. By 2002 these plots contained more *Trifolium pratense, Rumex acetosa and Anthriscus sylvestris* than they had in 2000. This pattern of the various treatments giving rise to more of these three species in 2002 than 2000 was seen in both of the other two treatments as well. This however was not a change to the species make up of the meadow as these species were also common in the control plots. *Ranunculus* sp. were most common in the control plots however in the ordinations of the other treatments it showed as being more common in the soil seed bank suggesting a reduction in cover in the three treatments. The reasons for this are unclear.

Following the second survey the perennial herbs in the plots of each treatment had re-established their dominance. In the initial survey, the components of the vegetation within the bare ground plots were more closely related to the soil seed bank than they were two years later. However the plots treated with farmyard manure in 2000 were not similar to the either the soil seed bank or the seed content of manure. This suggests that the larger quantity of bare ground produced when the turf was removed initially was filled to a certain extent by a seeds germinating from the soil seed bank. This supports the idea of the regeneration niche (Grubb 1977) which states that the extent of bare ground will be a factor in determining the species which fill the gap. Two years later this vegetation showed no differences from the control plots.

At Cockle Park the species poor sward found within the experimental plots did not increase in diversity due to the treatments received. Only one species which was not recorded in the first survey was found in the second one. This was *Rumex acetosa* one plant of which was found within a plot which had received farmyard manure from an intensively managed sward.

It is a recurrent theme in experiments investigating gap colonisation in grasslands that vegetative re-growth is the most important factor in colonising small (≤ 1 m^2) gaps (Thompson 2000). The results reported here clearly lend further weight to the importance of vegetative re-growth in maintaining the botanical diversity within hay meadow vegetation. The vegetation, which replaced the bare ground within the meadows, was not similar to the species make up of either the soil seed bank or the farmyard manure.

The application of farmyard manure did not increase the cover of *Poa trivialis* which was the main species found within manure samples. It is possible however that manure application could play a role in incorporation of seed into the soil seed bank. The vegetative spread of large perennial herbs into the gaps highlights the role that the gap creation is thought to play in mediating competition and structuring plant communities (Crawley and May 1987). Indeed Lavorel *et al.*, (1994) showed an increase in species richness of grassland following the creation of gaps within the sward. As the extent of bare ground in a sward is increased so is the opportunity for the less competitive species (Marriot *et al.*, 1997). The amount of bare ground produced by the farmyard manure plots was more than was seen in the control plots. It is therefore possible that the gap creating properties of the manure application could be important in the long-term maintenance of botanical diversity.

8.0 Discussion.

The work presented in this thesis was carried out within traditionally managed farms in the Pennines, northern England. The meadows on these farms are highly valued for their botanical diversity (Rodwell 1992; Ratcliffe 1977). The meadows are generally protected by various designations such as SSSI status as well as being a priority habitat within the Pennine Dales ESA scheme. This scheme aims to protect the few remaining examples of this once widespread vegetation type (Rodwell 1992). The scheme also has as a goal the enhancement of botanical diversity of meadows which have lost botanical interest due to the introduction of more intensive agricultural practices.

The meadows are refuges for a range of herbs and grasses which were, prior to the commencement of agricultural activity in the Pennines, likely to found within the native woodland of the area (Rodwell 1992). Species of perennial herb such as *Geranium sylvaticum, Sanguisorba officinalis, Geum rivale, Alchemilla vulgaris* agg., *Cirsium helenioides* as well as *Filipendula ulmaria, Trollius europaeus* and *Caltha palustris* in wetter areas are especially noteworthy as is the hemi-parasitic annual herb *Rhinanthus minor.*

Various aspects of meadow management such as hay cut date, grazing regime and inorganic fertiliser use have received a good deal of research attention and their influences on botanical diversity of meadows are well understood (Simpson, Hunter and Jefferson 1996). The use of farmyard manure on these meadows, which is an integral part of the yearly management, has received less attention. Whilst it is accepted that the application of large quantities of farmyard manure to grassland will increase productivity at the expense of botanical diversity (Williams 1978), an understanding of the role that the application of more modest amounts of farmyard manure plays in maintaining botanical diversity in these species rich meadows is unclear. The aim of this study has been to investigate the hypothesis that farmyard manure application is involved in the maintenance of botanical diversity through the distribution of viable seed to suitable germination niches created by the manure application.

If viable seeds are to be incorporated into the manure that is spread in the meadows, generally in the spring, it must first be found in the hay that is cut from the meadows the previous summer. In the first chapter of this thesis samples of hay were obtained from two different traditionally managed farms with species rich meadows in the Pennines. These were Piper Hole Farm in Ravenstonedale, Cumbria and New House Farm in Malham, Yorkshire. Germination of the seed within samples taken from these hay bales showed the range of species and the number of each species.

Although the vegetation within the meadows contain a reasonable proportion of *G. sylvaticum, S. officinalis* as well as other perennial herbs such as *F. ulmaria*, the seed in the hay samples taken from Piper Hole was dominated by *Poa trivialis* with other abundant species being the grasses *Lolium perenne, Dactylis glomerata, Poa pratensis, Holcus lanatus, Agrostis capillaris* and *Phleum pratensis*. Few species of dicotyledonous herb were found to be present in any significant quantities, and those that were present were species not noted for conservation value such as *Plantago lanceolata, Myosotis spp.* and *Cerastium fontanum*. A mean of 1615 viable seeds in each kg of hay were found, and 845 of these were *P. trivialis*.

The vegetation within the meadows at New House Farm contained high quantities of *R. minor, F. ulmaria, A. vulgaris* agg as well as *G. sylvaticum*, although less than at Piper Hole. The hay samples obtained from New House Farm were found to contain a much lower number of seeds. On average 375 viable seeds in each kg of hay germinated. *P. trivialis*, *Bellis perennis* and *P. lanceolata* were the dominant species in these samples with mean viable seeds kg^{-1} of 94.2, 92.4 and 90.9 respectively. Other species of grass such as *L. perenne, Bromus hordeaceus* and *Anthoxanthum odoratum* the *Myosotis* spp. were also present in reasonable quantities.

Whilst some of the species present in the hay samples from the two farms are different, the general pattern is that grasses especially *P. trivialis*, annual herbs and low growing perennial herbs made up the bulk of these samples. The perennial herbs of conservation interest are notable by their almost complete absence. Just two seeds of *C. helenioides* were found in all of the samples.

The reasons for differences between the two farms in terms of the quantity of seeds found in hay is unclear. Discussion with the farmers failed to identify any particular differences in the hay making processes used. Differential seed production between the sward is possible. It could be suggested that the dominance of *Bellis perennis* within the hay samples at New House points towards an earlier hay cutting date at that site. However hay cutting started later and finished later at New House than Piper Hole. However as samples were obtained in different years and the fact that New House Farm (~350 m) is at a higher altitude than Piper Hole (~260 m) cut date alone is unlikely to provide the explanation. The production of seed from grasslands differs between years and so this would appear to be the most likely cause of the differences seen.

In order for the viable seeds found within hay to be transferred back into the meadows with the farmyard manure they must be able to enter the bedding of the livestock to which the hay is fed. There are two main routes by which this could occur. Firstly the seeds may pass through the digestive tract of the livestock, mainly cattle and be egested still viable in the dung or the seed may fall directly onto the bedding from the feed racks. A wide range of species including grassland plants have been shown to be capable of surviving passage through the ruminant digestive system with their viability intact (Bulow-Olsen 1980; Welch 1985; Jones, Noguchi and Bunch 1991; Gardener *et al.,* 1993; Mt Pleasant and Schlather 1994; Andrews 1995; Malo and Suarez 1995; Ghassali *et al.,* 1998; Pakeman *et al.,* 1998; Rupende *et al.,* 1998; Pakeman *et al.,* 1999; Woldu and Saleem 2000), it has also been shown that the germinability of some species can be increased by such treatment (Malo and Suarez 1995b).

In order to test the ability of the seeds in hay to survive passage through the digestive system of cattle stock were fed the hay from which the samples were initially taken and the resultant dung was collected and the viable seed content determined by germination. In addition to this a laboratory experiment was conducted in which known quantities of 5 species *P. trivialis, A. odoratum, S. officinalis, F. ulmaria* and *Myosotis arvensis* were subjected to a three stage *in vitro* ruminant digestion process adapted from the methods of Ocumpaugh and Swakon (1993).

In the first experiment, the dung collected from animals fed hay made from meadows at Piper Hole and New House farms contained a great deal less viable seed than the hay from which it originated. On average 1 kg of dung from the animals fed hay from Piper Hole contained 63.28 viable seeds whereas 1 kg of the dung from animals fed the hay from New House Farm contained 32.6 viable seeds. The dung of animals fed hay from both farms was dominated by *P. trivilalis* with a much lower number of species germinating from the dung than was found within the hay. Both types of dung contained a number of other grass species and the range of herbs present were limited to *P. lanceolata* and *Myosotis* app. and *B. perennis* at New House. Notable in both types of dung was the presence of reasonable quantities of *Juncus effusus* and *J. bufonius*. It is hypothesised that the germinative capacity of these two species was increased by passage through the digestive system. *Juncus* spp. are known to have small seeds which are persistent in soil seed banks (Thompson, Bakker and Bekker 1997) and these characteristics have been shown to be also linked to the ability of seeds to be dispersed by endozoochorous processes. Pakeman *et al.*, (2002) showed that small seed size and the capacity to form a long lived seed bank were associated with those seeds dispersed via the digestive systems of sheep and rabbits in various UK grasslands whereas Gardiner *et al.*, (1993) found that smaller seeds were passed through the digestive system of cattle quicker than larger seeds and this helped them survive digestion intact.

The second experiment using *in vitro* digestive techniques showed however that the species *Myosotis arvensis* was most reduced in viability following the simulated digestive process despite it being found in the dung of animals fed hay from New House and Piper Hole. The small seed size of this species may account for these differing results. In cattle this species may pass out of the digestive system more quickly. Whilst all five of the species tested showed drastic reductions in viability following the three stage process all of species had at least a few seeds remain viable following digestion. Different responses to each individual stage of the digestive process were noticeable. Both of grass species were significantly reduced in viability following the initial seed abrasion to mimic chewing whilst *F. ulmaria* significantly increased in germination following abrasion.

Incubation in rumen fluid reduced viability in all the species tested, however *for A. odoratum* and *F. ulmaria* this decline only occurred after 48 and 72 hours respectively. Each of the other three species showed significant reductions in germination following only 12 hours of incubation.

The final step of the *in vitro* digestive process was incubation in acidified pepsin solution which represents post ruminal digestion. This step caused significant reductions in germination for all of the species especially *M. arvensis* which exhibited an almost total failure to germination following the 6 hour incubation period. *A. odoratum* however only showed a significant reduction in germination following pepsin incubation when it was preceded by incubation in rumen fluid for at least 48 hours.

These findings together show that the species present in the dung are generally just those species present in the largest quantities in the hay. All the species showed large reductions in viability following ruminant digestion both *in vivo* and *in vitro* with the post ruminal stage likely to be the most damaging. However species such *as S. officinialis* and *F. ulmaria* could if found in the hay in sufficient quantities be expected to be found within the dung produced by animals fed such hay.

In another part of Chapter 3 fresh manure from the barns at both farms was collected and its viable seed content determined following 0, 3, 6 and 12 months storage. The fresh manure samples from Piper Hole contained far more viable seed from more species than the equivalent dung samples. The manure obtained from Piper Hole contained 185 viable seeds kg^{-1} when fresh compared to 21.6 viable seeds kg^{-1} in the manure taken from New House Farm. The manure at New House Farm contained a similar number of seeds to the dung from that farm. This suggests that at Piper Hole much of the seed found within the manure is seed that has fallen directly out of the feed racks without passage through the digestive system, whilst at

New House Farm this is not the case. However, the low quantity of seed within the hay itself may have caused less reliable results in this case.

The Piper Hole manure samples, when fresh, were once again dominated by *P. trivialis* with other grasses and *Myosotis* spp and *P. lanceolata* also common. The species composition of seeds in these samples was very similar to the equivalent hay samples. Following 3 months of storage, the samples were largely unchanged, however 6 months of storage reduced the viability of all species and this effect was increased after 12 months storage. Once again the rush species *J. effusus* and *J. bufonius* became more prominent in samples stored for longer periods.

These two species of rush are common to wetter areas of grasslands (Clapham, Tutin and Warburg 1987) and as wet flushes are common in unimproved hay meadows (Rodwell 1992) it is likely that both species are constituents of the sward at Piper Hole despite not being recorded in the meadow vegetation survey. *J. effusus* was recorded however within the New House meadows.

The New House manure samples were also dominated by *P. trivialis* with *P. lanceolata* and *B. perennis* also prominent. Again following 3 months storage the species make up and quantity of seed was relatively unchanged however it was greatly reduced by 6 months storage and 12 months storage gave rise to no viable at all. Similarly to the Piper Hole samples *J. bufonius* and to a lesser extent *J. effusus* were characteristic species.

In addition to this survey of the seed content of manure an experiment was set up using known quantities of the same species of seed as described in the *in vitro* experiment above. These seeds were stored for 0, 3, 6, 12 months within a large manure heap at two different depths. Again the species *F. ulmaria* and *S. officinalis*, species not found in the samples of hay taken but present in the meadows, were investigated along with three other species common in both hay and manure samples. All species were reduced in germanative capacity following storage although some seeds of all species remained viable for 12 months storage. However for all species 6-12 months storage significantly reduced germination whilst 3 months storage only significantly reduced germination of *Myosotis arvensis*. Deeper storage further exacerbated this effect.

These experiments demonstrate that species of conservation interest found within Pennine meadows are unlikely to be spread between meadows by manure application. Hay from floristically rich meadows was shown to not contain many of the species present in the vegetation from which it is cut. Grass seeds, especially *P. trivialis* are most strongly represented in the hay. Those seeds which are found within the hay are unlikely to survive the digestive processes of the livestock. Any seed which does become incorporated into the manure without previous digestion must then survive the storage period of the manure, which if longer than 6 months, also has a drastic negative effect on germination. Whilst *P. trivialis* dominated the dung and manure samples this was mainly due to the large quantity of seed within the hay rather than to any differential survival during digestion or storage. Evidence suggests that species of perennial herb such as *S. officinalis* and *F. ulmaria* would survive digestion and storage at least as well if the species could initially be incorporated into the hay samples.

It is known that seed is shed from the sward after cutting, especially during the operations of hay turning (Smith, Pullan and Shiel 1996). Therefore, a large quantity of seed could be incorporated into the seed bank prior to any seed being removed into the hay. Also the manure when spread could be a factor in returning a large amount of seed to the soil when it is spread. In order to asses the role that farmyard manure application can play as a source of seed within meadows it was necessary gain and understanding of other sources of seed in the hay meadow system. The permanent and transient soil seed banks within meadows at both New House and Piper Hole were investigated to determine if they contained a similar range of species to the hay and manure samples taken from the two farms.

These results showed that the soil seed bank contains a range and quantity of species which is different from that exhibited in the vegetation above it. This is a characteristic that has been shown in a number of studies of various types of hay meadow (Kirkham and Kent 1997; Jensen 1998; Smith *et al.,* 2002). All of the species that were found within hay and manure samples in both farms were found within the soil seed bank at each farm. In addition to this there were a range of species with early flowering and seed setting habits such as *Rhinanthus minor* and *Alchemilla vulgaris* agg.. The later flowering perennial herbs species of conservation interest were largely absent or only found in very small quantities.

The samples of vegetation, soil seed bank and hay/manure samples at both farms were compared to a framework of National Vegetation Classification mesotrophic grassland sub-communities. The vegetation at Piper Hole was generally associated with the MG3a and the New House meadow vegetation was more variable but mostly associated with MG3a, 5a and 5b, whilst the soil seed banks from both sites were similar to MG6 sub-communities. These are species poor pasture communities dominated by grasses and a few perennial herbs such as *B. perennis P. lanceolata* and *Ranunculus acris*.

The species make up of the hay and manure from both farms was shown to be related mostly to MG7 sub-communities which are *Lolium perenne* dominated pastures. So whilst both farms had differing vegetation types the seed content of the soil seed banks were similar and the hay/manure samples even more so. These results suggest that whilst manure may play a role in the incorporation of seed to the soil seed bank seed may not have a significant role in maintaining botanical diversity within the meadows. The suggestion is that in order to maintain botanical diversity the vegetative reproduction of many of the distinctive species of plant within hay meadows is more important than reproduction by seed.

The final experiment in this thesis attempted to determine if the application of farmyard manure produced bare ground in meadow vegetation and, if so, was it a favourable site for the germination of seed from either the manure itself or the soil seed bank. The experiment was set up in a meadow at Piper Hole as well as being repeated on species poor grassland which had regenerated from intensive arable production since 1994 years at Cockle Park Farm, Northumberland. Whilst the experiment at Cockle Park was conducted as planned, restrictions imposed by the Foot and Moth disease during 2000 meant that results could not be collected until a year later at Piper Hole.

At both sites manure applied in the spring gave rise to gaps in the vegetation which were still apparent in the subsequent summer. At Cockle Park farm it was possible to examine the plots again in the subsequent summer. These results showed that no species found in the manure samples yet absent from the vegetation were incorporated into the sward. Examination of the plots at Piper Hole in 2002 showed that the vegetation present in plots which had been produced by gaps created by turf removal and manure application were not distinguishable from control plots. There was limited evidence that in the first summer gaps produced by turf removal were partially colonised by species similar to that found within the soil seed bank, however at the next sampling this distinction was not seen. The species which colonised these gaps include perennial herb species which are absent from the soil seed bank and from the manure which was applied. These results suggest that vegetative re-growth was important in determining the re-establishment of meadow vegetation in gaps.

8.1 Conclusions

The main findings of this study were therefore that during the years in which the hay and manure samples were collected from both New House Farm and Piper Hole, seed of the main species of conservation interest failed to germinate from the samples. The fresh manure samples were in general similar in species composition to the hay samples however storage of the manure for 6 months or more reduced the amount of seed present as well as the number of species present. This made these older manure samples similar to the dung samples in species composition.

These results suggest that the majority of the seeds present in manure do not pass through the digestive system of the cattle but rather fall directly into the bedding material. The majority of species present in hay and therefore in manure and dung samples were grasses and annual herbs. *Poa trivialis* dominated samples from both sites.

Evidence suggests however that if the perennial herbs *Filipendula ulmaria* and *Sanguisorba officinalis* were to be incorporated into the hay crop, through a later hay cut, there capability of surviving digestion by cattle and storage within a manure heap would at least as good as *P. trivialis*. The hay cut dates are extremely variable in the Pennines with occasionally very late hay cut dates (Smith and Jones 1991 and Rodwell and Dring 2001). It is during these years that the possibility arises that such plants could become incorporated into hay and subsequently into manure.

The soil seed banks at Piper Hole and New House farms contained the species present within manure samples as well as other species such as *Rhinanthus minor* and *Alchemilla vulgaris* agg. which are early flowering species within these meadows. The seed banks contained a higher density of seed than the manure samples. However the soil seed banks were distinctly different in species make up from the vegetation being more comparable to species poor pastures. Again the main species of conservation interest were largely absent from the soil seed bank or only present in small quantities and at very low frequencies. Again sampling the seed bank, including the transient seed bank as was done here, in the year after later hay cuts would enable the full role of seed in the life cycle of these perennial plants to be understood.

The application of manure onto meadow vegetation produced bare ground that was filled by vegetation similar to the surrounding meadow vegetation. Species found to be absent or only occasional in the seed bank and absent from the manure were amongst those that colonised these gaps. Unless seed from perennial herbs drop prior to or during the hay cutting process and germinate immediately this suggests vegetative spread of these plants is an important factor in the survival of these species within the sward. For the perennial herb such *Geranium sylvaticum* this would seem unlikely. There is good evidence that this plant requires cold treatment to germinate (Hill 2001) and Smith, Shiel and Pullan (1996) found very little seed of this species dropping from the sward when the hay making process was simulated with a crop of herbage from a traditionally managed species rich sward containing a high quantity of the species in the sward.

8.1.1 Implications of main findings

The ability of species of high conservation value within Pennine hay meadows to colonise gaps within the sward and to re-establish vegetation which cannot be differentiated from the previous vegetation highlights the reduced role that seed may play within extant hay meadows. The perennial nature of these species allows them to survive and reproduce vegetatively for a number of years. The role that unusually late hay cuts which allow these species to produce seed which could enter the soil seed bank and/or the hay (and subsequently manure), play in the long term dynamics of hay meadows has not been studied. It is possible that the frequency of these later hay cuts are more important than the average hay cut date each year.

The re-creation of hay meadow vegetation is usually marked by a limit to species diversity caused by lack of available propagules (Bakker *et al.,* 1996). Management for increased species richness may well therefore require differing management than is necessary to maintain a species rich sward which may be far less reliant on seed production within the ecosystem. Such differences should be taken into consideration in the drawing up of agreements in agri-environment schemes.

Whilst the successful investigation of hay strewing in increasing botanical diversity within meadows has been reported (Wells, Frost and Bell 1986; Mortimer *et al.,* 2002), these studies have taken place in the southern part of England. These studies have occurred on distinctively differing vegetation types. The meadows in the Pennines contain a different range of species. Later flowering perennial herbs are far more characteristic of Pennine meadows than their lowland counterparts. Much of this is probably due to the harsher environment found in the Pennines. The shorter growing season this brings about may give less opportunity to produce ripe seeds, due to the necessity of obtaining a hay crop and a crop for aftermath grazing within this short time. This may in turn give rise to species less reliant on seed to maintain the community structure and give a greater emphasis on vegetative processes.

Even within the same species, differing phenologies in different parts of the country may highlight this. For example Mortimer *et al.,* (2002) found that in the Chilterns in southern England *Centaurea nigra* was a species particularly favoured by hay strewing. However Smith, Pullan and Shiel (1996) found that in a meadow in the Pennines this species was not found with ripe seed even by 21st August. It would therefore be unlikely to produce ripe seed in all but the very latest years. Nevertheless it is a common species within Pennine hay meadows. None of this species was found as seed in hay/manure samples taken nor was it found within the soil seed bank.

8.2 Further Work

8.2.1 In addition to this study

The possibility of different responses of meadow vegetation to gaps produced by manure application in the autumn or winter could be investigated. Differing species germinate in the autumn compared to the spring (Grime, Hodgson and Hunt 1988). Indeed Wells, Frost and Bell (1986) showed that cattle dung patches produced in the spring were colonised more quickly than those produced in the autumn. These could therefore alter the species colonising the patches. It may be expected that vegetative growth into patches may occur quicker in the spring and so autumn/winter produced patches may be more suitable for seed germination.

Knowledge of the contribution of the seed rain during the period of grass growth as well as during the hay cutting period would enable a clearer picture of the role the seed plays in the maintenance of botanical diversity in meadows. Work on this was due to be carried out in this study during the summer period of 2001 but due to the restrictions imposed by the foot and mouth outbreak this was not possible. It was planned that seed traps would be buried at random within the meadows and the contents emptied and identified by germination weekly throughout the crop growing phase as well as during the hay making process. It was also hoped that it would have been possible to place seed traps along a transect away from the species rich sward where it neighboured a species poor sward to see if any seed dispersed into neighbouring fields.

The influence that unusually late hay cuts play in the dynamics of Pennine meadow systems warrants investigation. In those years it will become possible for those species not found in anything but small quantities within the soil seed bank to enter the soil in much larger quantities as well as the manure that is spread onto the meadow at a later date. It is possible that without these years arising changes in the relative abundance and even species make up of the meadows could occur.

By tracking the production and fate of seeds in such years the role that is played by seed in such years would be elucidated. In addition to this knowledge of the ecology of key individual species in relation to there life histories in Pennine hay meadows in particular the average lifespan of such species would enable more reliable minimum requirements for there survival in the sward to be ensured. For example, the half life of adult perennial grassland species has been shown to vary from 1-2 years up to more than 50 years (Harper 1967; Tamm 1972a,b Sarukhan and Harper 1973) information such as this may be required for Pennine meadow plants in order to determine a minimum requirement for unusually later hay cuts.

8.2.2 In relation to the wider context of hay meadows.

As restorations schemes are often seed limited (Bakker and Berendse 1999) the role that seed dispersal plays in meadows is of great importance. Environmentally Sensitive Area payments for Tier Two hay meadow vegetation require the occurrence of a certain level of species diversity within the sward. This is often found in corners of otherwise more species poor swards. The dispersal of seeds throughout the rest of the meadow is therefore necessary to restore species richness. A thorough understanding of seed dispersal within meadows is therefore necessary.

As already stated, the use of hay strewing to increase botanical diversity has been reported in a number of studies (Wells, Frost and Bell 1986; Atkinson *et al.*, 1995; Jones *et al.*, 1995; Mortimer *et al.*, 2002). It has not however been attempted with the distinctive vegetation present in Pennine hay meadows which contain a larger number of late flowering perennial herbs than lowland grassland types. Given the reliance on vegetative reproduction this study points towards, it is likely that such methods will not prove effective for this vegetation type.

The dispersal of seed is also of great importance in understanding the effects of the isolation of these plant communities in the wider countryside. If the regeneration and maintenance of these plant communities rely on seed input from other sources such as neighbouring hay meadows, roadside verges and woodland remnants for example the targeting of restoration schemes should be at areas which contain a large enough quantity of these features.

The dispersal of meadow plants over larger distances is an area of research that is currently lacking and needs attention. Few studies have addressed these issue directly (Poschlod and WallisDeVries 2002). The study of hay meadow vegetation needs to be expanded to take a wider view so that meadows are not just considered in isolation.

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10. Appendix.

1. **List of species and percentage cover in each quadrat at Piper Hole Farm.**

Lime Kiln 1

A		B		C		D	
Sanguisorba officinalis	30	*Anthoxanthum odoratum*	35	*Sanguisorba officinalis*	40	*Anthoxanthum odoratum*	20
Ranunculus repens	30	*Plantago lanceolata*	30	*Geranium sylvaticum*	40	*Holcus lanatus*	20
Anthoxanthum odoratum	25	*Sanguisorba officinalis*	30	*Anthoxanthum odoratum*	20	*Geranium sylvaticum*	20
Geranium sylvaticum	20	*Ranunculus repens*	20	*Rhinanthus minor*	10	*Sanguisorba officinalis*	10
Rhinanthus minor	20	*Holcus lanatus*	20	*Alchemilla vulgaris* agg.	10	*Ranunculus repens*	5
Phleum pratense	5	*Cynosurus cristatus*	10	*Cynosurus cristatus*	10	*Lolium perenne*	2
Lolium perenne	5	*Filipendula ulmaria*	5	*Holcus lanatus*	5	*Plantago lanceolata*	2
Cynosurus cristatus	5	*Lolium perenne*	5	*Bromus hordeaceus*	2	*Poa trivialis*	2
Anthriscus sylvestris	5	*Trifolium pratense*	5	*Plantago lanceolata*	2	*Rhinanthus minor*	2
Holcus lanatus	1	*Myostis discolor*	5	*Trifolium repens*	2	*Filipendula ulmaria*	2
Bromus hordeaceus	1	*Cerastium fontanum*	5	*Phleum pratense*	1	*Cirsium helenioides*	2
Poa annua	1	*Bromus hordeaceus*	1	*Lolium perenne*	1	*Phleum pratense*	1
Plantago lanceolata	1	*Poa trivialis*	1	*Poa pratense*	1	*Cynosurus cristatus*	1
Rumex acetosa	1	*Poa pratense*	1	*Dactylis glomerata*	1	*Bromus hordeaceus*	1
Trifolium pratense	1	*Rumex acetosa*	1	*Rumex acetosa*	1	*Alopecurus pratensis*	1
Bellis perennis	1	*Trifolium repens*	1	*Anthriscus sylvestris*	1	*Agrostis capillaris*	1
		Rhinanthus minor	1			*Rumex acetosa*	1
		Bellis perennis	1			*Trifolium pratense*	1
						Bellis perennis	1
						Myostis discolor	1
						Cerastium fontanum	1

Alchemilla vulgaris agg.	1
Taraxacum officinale agg.	1
Conopodium majus	1
Lathyrus pratensis	1

Lime Kiln 2

	A		B		C		D
Filipendula ulmaria	40	*Geranium pratense*	40	*Filipendula ulmaria*	30	*Geranium sylvaticum*	35
Rhinanthus minor	20	*Heracleum sphondylium*	10	*Anthoxanthum odoratum*	5	*Sanguisorba officinalis*	5
Anthoxanthum odoratum	20	*Plantago lanceolata*	10	*Poa trivialis*	5	*Lolium perenne*	5
Ranunculus repens	5	*Rhinanthus minor*	10	*Rumex acetosa*	5	*Plantago lanceolata*	5
Plantago lanceolata	5	*Anthoxanthum odoratum*	5	*Stellaria media*	5	*Trifolium pratense*	2
Holcus lanatus	2	*Holcus lanatus*	2	*Lolium perenne*	2	*Rhinanthus minor*	2
Cynosurus cristatus	2	*Rumex acetosa*	2	*Geranium pratense*	2	*Ranunculus repens*	2
Geranium sylvaticum	2	*Sanguisorba officinalis*	2	*Ranunculus repens*	2	*Anthoxanthum odoratum*	2
Poa trivialis	2	*Filipendula ulmaria*	2	*Festuca pratensis*	2	*Holcus lanatus*	2
Anthriscus sylvestris	2	*Lolium perenne*	1	*Phleum pratense*	1	*Poa trivialis*	2
Lolium perenne	1	*Poa annua*	1	*Conopodium majus*	1	*Filipendula ulmaria*	2
Poa pratense	1	*Ranunculus repens*	1	*Bromus hordeaceus*	1	*Anthriscus sylvestris*	1
Festuca pratensis	1	*Festuca pratensis*	1	*Alopecurus pratensis*	1	*Bellis perennis*	1
Rumex acetosa	1			*Anthriscus sylvestris*	1	*Myostis discolor*	1
Bellis perennis	1					*Cerastium fontanum*	1
Cerastium fontanum	1					*Taraxacum officinale* agg.	1
Taraxacum officinale agg.	1					*Conopodium majus*	1
Conopodium majus	1					*Rumex acetosa*	1

Heracleum sphondylium	1					*Dactylis glomerata*	1
						Festuca pratensis	1
						Alopecurus pratensis	1
						Agrostis capillaris	1
						Cynosurus cristatus	1
						Bromus hordeaceus	1

Lim Kiln 3

	A		B		C		D
Plantago lanceolata	40	*Ranunculus repens*	20	*Centaurea nigra*	55	*Geranium sylvaticum*	35
Sanguisorba officinalis	30	*Plantago lanceolata*	20	*Sanguisorba officinalis*	40	*Trifolium pratense*	10
Holcus lanatus	5	*Filipendula ulmaria*	20	*Ranunculus repens*	10	*Sanguisorba officinalis*	5
Lolium perenne	5	*Cirsium helenioides*	20	*Taraxacum officinale* agg.	10	*Plantago lanceolata*	5
Cynosurus cristatus	5	*Geranium pratense*	15	*Anthoxanthum odoratum*	5	*Anthoxanthum odoratum*	2
Anthoxanthum odoratum	5	*Anthoxanthum odoratum*	5	*Lolium perenne*	5	*Lolium perenne*	2
Rumex acetosa	5	*Holcus lanatus*	5	*Cynosurus cristatus*	5	*Cynosurus cristatus*	2
Trifolium pratense	3	*Lolium perenne*	5	*Plantago lanceolata*	5	*Poa trivialis*	2
Rhinanthus minor	3	*Cynosurus cristatus*	5	*Rumex acetosa*	5	*Rumex acetosa*	2
Bromus hordeaceus	2	*Poa trivialis*	5	*Trifolium pratense*	3	*Rhinanthus minor*	2
Poa trivialis	1	*Trifolium pratense*	3	*Rhinanthus minor*	3	*Ranunculus repens*	2
Poa annua	1	*Rhinanthus minor*	3	*Bellis perennis*	3	*Bellis perennis*	2
Ranunculus repens	1	*Bellis perennis*	3	*Cirsium helenioides*	2	*Holcus lanatus*	2
Bellis perennis	1	*Festuca pratensis*	2	*Holcus lanatus*	2	*Alopecurus pratensis*	1
Myostis discolor	1	*Agrostis capillaris*	1	*Poa trivialis*	2	*Agrostis capillaris*	1
Cerastium fontanum	1	*Rumex acetosa*	1	*Agrostis capillaris*	2	*Phleum pratense*	1
Taraxacum officinale agg.	1	*Geranium sylvaticum*	1	*Myostis discolor*	1		
Euphrasia nemorosa	1	*Lathyrus pratensis*	1	*Cerastium fontanum*	1		

		Euphrasia nemorosa	1	Lathyrus pratensis	1		
				Bromus hordeaceus	1		

PH Field (6)

A		B		C		D	
Plantago lanceolata	45	*Geranium sylvaticum*	55	*Geranium sylvaticum*	40	*Geranium sylvaticum*	35
Ranunculus acris	20	*Anthoxanthum odoratum*	20	*Bromus hordeaceus*	40	*Anthoxanthum odoratum*	10
Filipendula ulmaria	15	*Cynosurus cristatus*	10	*Plantago lanceolata*	10	*Holcus lanatus*	5
Taraxacum officinale agg.	10	*Bellis perennis*	5	*Rumex acetosa*	10	*Bromus hordeaceus*	5
Anthoxanthum odoratum	5	*Taraxacum officinale* agg.	5	*Anthoxanthum odoratum*	10	*Poa trivialis*	5
Holcus lanatus	5	*Plantago lanceolata*	5	*Lolium perenne*	10	*Plantago lanceolata*	5
Cynosurus cristatus	3	*Holcus lanatus*	5	*Poa trivialis*	5	*Rumex acetosa*	5
Rumex acetosa	3	*Lolium perenne*	5	*Agrostis capillaris*	5	*Sanguisorba officinalis*	5
Sanguisorba officinalis	3	*Bromus hordeaceus*	5	*Trifolium pratense*	5	*Ranunculus repens*	5
Bellis perennis	2	*Rumex acetosa*	5	*Ranunculus repens*	5	*Bellis perennis*	2
Bromus hordeaceus	2	*Ranunculus repens*	3	*Taraxacum officinale* agg.	3	*Agrostis capillaris*	2
Lolium perenne	1	*Myostis discolor*	3	*Sanguisorba officinalis*	2	*Trifolium pratense*	2
Rhinanthus minor	1	*Rhinanthus minor*	2	*Bellis perennis*	2	*Rhinanthus minor*	2
Anthriscus sylvestris	1	*Sanguisorba officinalis*	2	*Filipendula ulmaria*	2	*Lolium perenne*	2
Myostis discolor	1	*Trifolium pratense*	2	*Stellaria media*	2.	*Cynosurus cristatus*	1
Lathyrus pratensis	1	*Trifolium repens*	1	*Vicia sativa*	1	*Anthriscus sylvestris*	1
Centaurea nigra	1	*Anthriscus sylvestris*	1	*Holcus lanatus*	1	*Filipendula ulmaria*	1
		Cerastium fontanum	1	*Cynosurus cristatus*	1	*Myostis discolor*	1
		Conopodium majus	1			*Cerastium fontanum*	1
		Vicia sativa	1			*Taraxacum officinale* agg.	1
						Stellaria media	1

Lathyrus pratensis	1
Vicia sativa	1

Football pitch

A		B		C		D	
Caltha palustris	40	*Holcus lanatus*	30	*Plantago lanceolata*	35	*Poa trivialis*	30
Bromus hordeaceus	20	*Bromus hordeaceus*	20	*Trifolium pratense*	35	*Holcus lanatus*	5
Anthoxanthum odoratum	20	*Poa trivialis*	20	*Taraxacum officinale* agg.	20	*Lolium perenne*	5
Ranunculus repens	5	*Heracleum sphondylium*	10	*Vicia sativa*	10	*Bromus hordeaceus*	5
Filipendula ulmaria	5	*Lolium perenne*	5	*Rumex acetosa*	5	*Plantago lanceolata*	5
Holcus lanatus	3	*Rumex acetosa*	5	*Trifolium repens*	5	*Sanguisorba officinalis*	5
Lolium perenne	3	*Sanguisorba officinalis*	3	*Anthoxanthum odoratum*	3	*Ranunculus repens*	5
Poa trivialis	3	*Bellis perennis*	3	*Lolium perenne*	3	*Bellis perennis*	5
Rumex acetosa	3	*Agrostis capillaris*	2	*Agrostis capillaris*	3	*Taraxacum officinale* agg.	5
Festuca pratensis	2	*Ranunculus repens*	2	*Bellis perennis*	3	*Trifolium pratense*	5
Myostis discolor	2	*Filipendula ulmaria*	2	*Myostis discolor*	3	*Anthoxanthum odoratum*	2
Cerastium fontanum	2	*Cerastium fontanum*	2	*Cerastium fontanum*	3	*Agrostis capillaris*	2
Bellis perennis	2	*Taraxacum officinale* agg.	2	*Arrhenatherum elatius*	2	*Rumex acetosa*	2
Alopecurus pratensis	1	*Arrhenatherum elatius*	1	*Rhinanthus minor*	2	*Myostis discolor*	2
Agrostis capillaris	1	*Trifolium pratense*	1	*Ranunculus repens*	2	*Festuca pratensis*	1
		Anthriscus sylvestris	1	*Holcus lanatus*	2	*Alopecurus pratensis*	1
		Myostis discolor	1	*Cynosurus cristatus*	1	*Phleum pratense*	1
				Bromus hordeaceus	1	*Arrhenatherum elatius*	1
				Poa annua	1	*Filipendula ulmaria*	1
						Cerastium fontanum	1

Great Bottom	A		B		C
Sanguisorba officinalis	40	*Anthriscus sylvestris*	30	*Filipendula ulmaria*	40	*Holcus lanatus*	30
Lolium perenne	20	*Holcus lanatus*	10	*Lolium perenne*	20	*Bromus hordeaceus*	20
Plantago lanceolata	20	*Trifolium pratense*	10	*Bromus hordeaceus*	20	*Anthoxanthum odoratum*	20
Trifolium pratense	10	*Geranium sylvaticum*	10	*Poa trivialis*	20	*Lolium perenne*	10
Bellis perennis	10	*Anthoxanthum odoratum*	10	*Plantago lanceolata*	10	*Poa trivialis*	5
Anthoxanthum odoratum	5	*Bromus hordeaceus*	5	*Rumex acetosa*	10	*Plantago lanceolata*	5
Holcus lanatus	5	*Poa trivialis*	5	*Anthoxanthum odoratum*	5	*Trifolium pratense*	3
Bromus hordeaceus	3	*Plantago lanceolata*	5	*Phleum pratense*	3	*Sanguisorba officinalis*	3
Agrostis capillaris	3	*Bellis perennis*	3	*Holcus lanatus*	3	*Taraxacum officinale* agg.	3
Rumex acetosa	3	*Vicia sativa*	3	*Cynosurus cristatus*	3	*Rumex acetosa*	2
Myostis discolor	3	*Lolium perenne*	2	*Bellis perennis*	3	*Ranunculus repens*	2
Poa annua	2	*Myostis discolor*	2	*Agrostis capillaris*	2	*Bellis perennis*	2
Geranium pratense	2	*Cynosurus cristatus*	1	*Anthriscus sylvestris*	2	*Myostis discolor*	2
Ranunculus repens	2	*Agrostis capillaris*	1	*Myostis discolor*	2	*Cerastium fontanum*	2
Phleum pratense	2	*Ranunculus acris*	1	*Cerastium fontanum*	2	*Phleum pratense*	1
Anthriscus sylvestris	1	*Cerastium fontanum*	1	*Taraxacum officinale* agg.	2	*Alopecurus pratensis*	1
Dactylis glomerata	1	*Alchemilla vulgaris* agg.	1	*Ranunculus repens*	1	*Agrostis capillaris*	1
		Taraxacum officinale agg.	1	*Alopecurus pratensis*	1	*Anthriscus sylvestris*	1
						Conopodium majus	1
						Stellaria media	1
						Vicia sativa	1

Appendix 2 Piper Hole Hay seed kg^{-1}.

	PH 1	PH 2	PH 3	PH 4	PH 5
Lolium perenne	88.78	440.24	296.77	35.44	31.52
Dactlylis glomerata	29.59	68.29	193.55	407.59	25.00
Poa trivialis	891.84	715.85	1510.75	159.49	948.91
Poa pratensis	126.53	56.10	205.38	29.11	168.48
Poa annua	0.00	14.63	3.23	0.00	4.35
Holcus lanatus	2.04	20.73	19.35	177.22	1.09
Alopecurus pratensis	22.45	8.54	7.53	29.11	18.48
Phleum pratensis	30.61	63.41	52.69	83.54	17.39
Cynosurus cristatus	0.00	0.00	2.15	16.46	3.26
Bromus hordeaceus	9.18	17.07	27.96	0.00	8.70
Anthoxanthum odoratum	15.31	17.07	29.03	16.46	2.17
Deschampsia cespitosa	12.24	0.00	0.00	1.27	0.00
Agrostis capillaris	87.76	50.00	68.82	39.24	33.70
Agrostis stonlonifera	0.00	0.00	0.00	5.06	0.00
Arrehenatharum elatius	0.00	0.00	0.00	1.27	9.78
Festuca arundinacea	1.02	0.00	0.00	0.00	0.00
Festuca pratensis	1.02	0.00	1.08	0.00	0.00
Juncus effusus	2.04	0.00	3.23	10.13	4.35
Juncus bufonious	0.00	0.00	4.30	0.00	4.35
Polygonum persicaria	4.08	1.22	7.53	0.00	7.61
Polygonim bistorta	2.04	2.44	3.23	1.27	2.17
Plantago lanceolata	4.08	131.71	29.03	29.11	0.00
Rumex acetosa	2.04	3.66	3.23	25.32	16.30
Cerastium fontanum	12.24	10.98	9.68	18.99	2.17
Myostis discolor	22.45	32.93	7.53	6.33	25.00
Ajuga reptans	1.02	1.22	0.00	0.00	0.00

Stellaria media	1.02	0.00	0.00	1.27	0.00
Urtica dioica	0.00	0.00	0.00	37.97	0.00
Chamerion angustifolium	0.00	0.00	0.00	1.27	0.00
Montia fontanum	6.12	0.00	0.00	0.00	8.70
Bellis perennis	13.27	12.20	15.05	3.80	1.09
Ranunculus sp	20.41	0.00	0.00	5.06	3.26
Veronica chamaedrys	1.02	0.00	0.00	0.00	0.00
Cirsium helenioides	2.04	0.00	0.00	0.00	0.00
Vicia sepium	2.04	0.00	0.00	0.00	0.00
Trifolium sp.	1.02	0.00	1.08	0.00	0.00
total	1415.31	1668.29	2502.15	1141.77	1347.83

Appendix 3 Seed kg^{-1} of each species in individual manure samples from Piper Hole.

fresh	kg-1 A		kg-1 B	3 month old	kg-1 A		kg-1 B
Poa trivialis	144.6	*Poa trivialis*	114.2	Poa trivialis	139.4	Poa trivialis	122.1
Poa pratensis	16.0	*Lolium perenne*	27.1	Lolium perenne	10.3	Lolium perenne	31.0
Lolium perenne	13.1	*Poa pratensis*	18.1	Juncus bufonius	4.8	Poa pratensis	12.4
Myostis discolor	4.0	*Bromus hordeaceus*	2.6	*Bromus hordeaceus*	2.4	Juncus bufonius	5.5
Juncuc bufonious	2.9	*Dactylis glomerata*	2.6	*Agrostis capillaris*	1.8	*Rannunculus sp*	4.8
Hordeum sp	2.3	*Plantago lanceolata*	2.6	*Cerastium fontanum*	1.2	*Plantago lanceolata*	2.8
Dactylis glomerata	1.7	*Myostis discolor*	1.9	*Holcus lanatus*	1.2	*Myostis discolor*	1.4
Juncus effusus	1.7	*Anthoxanthum odoratum*	3.2	*Plantago lanceolata*	1.2	*Alopecurus pratensis*	1.4
Festuca pratensis	1.1	*Cerastium fontanum*	1.3	*Myostis discolor*	1.2	*Phleum pratensis*	1.4
Plantago lanceolata	1.1	*Rannunculus sp*	0.6	*Ruemx acetosa*	0.6	*Anthoxanthum odoratum*	0.7
Poa annua	0.6	Hordeum sp	0.6	*Taraxacum officinale*	0.6	*Juncus effusus*	0.7

				agg			
Rannunculus sp	0.6	*Phleum pratensis*	0.6	Poa pratensis	0.6	*Stellaria media*	0.7
Bromus hordeaceus	0.6	*Agrostis capillaris*	0.6			*Montia fontanum*	0.7
Holcus lanatus	0.6	*Trifolium pratense*	0.6			*Trifolium sp*	0.7
Phleum pratensis	0.6					*Cirsium arvensis*	0.7
Agrostis capillaris	0.6						
Rumex acetosa	0.6						
	192.6		176.8		165.5		186.9
6 month old	kg-1 A		kg-1 B	1 year old	kg-1 A		kg-1 B
Poa trivialis	115.7	Poa trivialis	116.2	Poa trivilis	19.1	Poa trivilais	22.1
Lolium perenne	1.7	*Ruemx acetosa*	1.5	Juncus bufonius	2.7	Juncus effusus	1.1
Trifolium sp	1.7	Juncus bufonius	1.5	Plantago lanceolata	1.8		
Rannunculus sp	0.9	Chamerion angustifolium	1.5	Lolium perenne	0.9		
		Poa pratensis	0.8	Rumex acetosa	0.9		
		Trifolium sp	0.8				
Total	120.0		122.3		25.5		23.2

Appendix 4 Seed kg^{-1} of each species within each sample of dung from animals fed hay from Piper Hole Farm.

	seed kg-1 Dung 1a		Dung 1b
Poa trivialis	13.5	*Poa trivialis*	24.2
Plantago lanceolata	14.1	*Lolium perenne*	3.6
Poa pratensis	7.1	*Juncus bufonious*	6.1
Lolium perenne	4.7	*Juncus effusus*	3.6
Juncus bufonious	2.4	*Poa pratensis*	2.4
Juncus effusus	1.8	*Plantago lanceolata*	1.8
Polygonum persicaria	1.2	*Polygonum persicaria*	0.6
Rumex acetosa	0.6	*Rumex acetosa*	0.6
Dactylis glomerata	0.6	*Dactylis glomerata*	0.6
Chamerion angustifolium	0.6	*Myostis discolor*	0.6
		Ceastium fontanum	0.6
Total	46.5		44.8

	Dung 2a		Dung 2b
Poa trivialis	50.0	*Poa trivialis*	53.1
Juncus bufonious	4.7	*Lolium perenne*	3.1
Poa pratensis	3.9	*Plantago lanceolata*	2.3
Lolium perenne	1.6	*Juncus effusus*	2.3
Plantago lanceolata	1.6	*Agrostis capillaris*	2.3
Polygonum persicaria	1.6	*Polygonum persicaria*	1.5
Agrostis capillaris	1.6	*Poa pratensis*	1.5
Trifolium sp.	0.8	*Trifolium sp.*	1.5
		Juncus bufonious	0.8
		Myostis discolor	0.8
		Polygonum bistorta	0.8
Total	65.6		70.0

	Dung 3a		Dung 3b
Poa trivialis	46.7	*Poa trivialis*	36.8
Poa pratensis	17.8	*Poa pratensis*	4.2

Juncus bufonious	8.9	*Plantago lanceolata*	2.1
Juncus effusus	8.9	*Juncus bufonious*	2.1
Polygonum persicaria	4.4	*Juncus effusus*	2.1
Chamerion angustifolium	3.3	*Agrostis capillaris*	2.1
Plantago lanceolata	3.3	*Ceastium fontanum*	1.1
Myostis discolor	2.2	*Rumex acetosa*	1.1
Lolium perenne	1.1	*Myostis discolor*	1.1
Rumex acetosa	1.1	*Cardamine flexuosa*	1.1
Ceastium fontanum	1.1		
Total	98.9		53.7

Appendix 5. List of species and percentage cover in each quadrat at New House Farm.

Big Meadow

	1		2		3		4
Anthoxanthum odoratum	30	*Anthoxanthum odoratum*	20	*Anthoxanthum odoratum*	10	*Anthoxanthum odoratum*	30
Alopecurus pratensis	10	*Alopecurus pratensis*	10	*Alopecurus pratensis*	2	*Alopecurus pratensis*	2
Cynosurus cristatus	3	*Holcus lanatus*	5	*Cynosurus cristatus*	5	*Holcus lanatus*	1
Bromus hordeaceus	5	*Lolium perenne*	2	*Bromus hordeaceus*	40	*Cynosurus cristatus*	3
Poa trivialis	5	*Cynosurus cristatus*	3	*Poa trivialis*	4	*Bromus hordeaceus*	40
Festuca rubra	10	*Bromus hordeaceus*	5	*Phleum pratense*	2	*Poa trivialis*	2
Phleum pratense	10	*Festuca pratensis*	2	*Plantago lanceolata*	10	*Dactylis glomerata*	1
Agrostis capillaris	1	*Festuca rubra*	2	*Rumex acetosa*	60	*Festuca rubra*	1
Plantago lanceolata	10	*Plantago lanceolata*	60	*Rhinanthus minor*	1	*Agrostis capillaris*	2
Rumex acetosa	10	*Rumex acetosa*	20	*Ranunculus repens*	20	*Plantago lanceolata*	30
Trifolium pratense	30	*Trifolium pratense*	30	*Ranunculus ficaria*	1	*Rumex acetosa*	10
Geranium sylvaticum	5	*Geranium sylvaticum*	5	*Anthriscus sylvestris*	5	*Geranium sylvaticum*	10
Rhinanthus minor	65	*Rhinanthus minor*	10	*Myostis discolor*	2	*Rhinanthus minor*	2
Sanguisorba officinalis	5	*Ranunculus acris*	10	*Conopodium majus*	5	*Ranunculus repens*	5
Ranunculus acris	40	*Bellis perennis*	40	*Stellaria media*	1	*Ranunculus ficaria*	1
Bellis perennis	30	*Taraxacum officinale* agg.	20	*Veronica chamaedrys*	1	*Anthriscus sylvestris*	5
Myostis discolor	1	*Conopodium majus*	5			*Bellis perennis*	30
Cerastium fontanum	5	*Stellaria media*	2			*Filipendula ulmaria*	10
Taraxacum officinale agg.	10	*Veronica chamaedrys*	1			*Myostis discolor*	2
Conopodium majus	5					*Alchemilla vulgaris* agg.	1
Centaurea nigra	1					*Taraxacum officinale* agg.	10
Veronica chamaedrys	1					*Conopodium majus*	5
						Euphrasia nemorosa	2

						Veronica chamaedrys	1
	5		**6**		**7**		**8**
Anthoxanthum odoratum	20	*Anthoxanthum odoratum*	5	*Anthoxanthum odoratum*	5	*Anthoxanthum odoratum*	20
Holcus lanatus	2	*Holcus lanatus*	2	*Alopecurus pratensis*	5	*Alopecurus pratensis*	5
Lolium perenne	1	*Cynosurus cristatus*	3	*Cynosurus cristatus*	3	*Lolium perenne*	1
Cynosurus cristatus	3	*Bromus hordeaceus*	10	*Bromus hordeaceus*	5	*Cynosurus cristatus*	1
Bromus hordeaceus	20	*Dactylis glomerata*	1	*Dactylis glomerata*	1	*Bromus hordeaceus*	25
Poa trivialis	5	*Festuca rubra*	2	*Festuca pratensis*	1	*Poa trivialis*	5
Festuca rubra	1	*Juncus effusus*	1	*Phleum pratense*	5	*Dactylis glomerata*	1
Phleum pratense	5	*Plantago lanceolata*	30	*Agrostis capillaris*	1	*Festuca pratensis*	1
Plantago lanceolata	10	*Rumex acetosa*	5	*Plantago lanceolata*	10	*Phleum pratense*	2
Rumex acetosa	10	*Trifolium pratense*	10	*Rumex acetosa*	10	*Carex nigra*	1
Trifolium pratense	10	*Rhinanthus minor*	20	*Trifolium pratense*	20	*Plantago lanceolata*	20
Rhinanthus minor	5	*Sanguisorba officinalis*	1	*Geranium sylvaticum*	5	*Rumex acetosa*	30
Ranunculus repens	10	*Ranunculus repens*	20	*Rhinanthus minor*	20	*Trifolium pratense*	50
Bellis perennis	40	*Ranunculus ficaria*	3	*Sanguisorba officinalis*	1	*Rhinanthus minor*	30
Filipendula ulmaria	30	*Bellis perennis*	30	*Ranunculus repens*	30	*Sanguisorba officinalis*	5
Myostis discolor	1	*Filipendula ulmaria*	5	*Bellis perennis*	20	*Ranunculus acris*	30
Alchemilla vulgaris agg.	5	*Myostis discolor*	1	*Filipendula ulmaria*	5	*Bellis perennis*	20
Taraxacum officinale agg.	5	*Taraxacum officinale* agg.	5	*Cerastium fontanum*	1	*Taraxacum officinale* agg.	10
Cirsium helenioides	30	*Stellaria media*	2	*Alchemilla vulgaris* agg.	5	*Conopodium majus*	5
		Cirsium helenioides	40	*Veronica chamaedrys*	1		
		Veronica chamaedrys	1				

Middle Meadow

	9		**10**		**1**		**2**

Anthoxanthum odoratum	5	*Anthoxanthum odoratum*	20	*Anthoxanthum odoratum*	5	*Anthoxanthum odoratum*	2
Alopecurus pratensis	5	*Alopecurus pratensis*	5	*Alopecurus pratensis*	1	*Holcus lanatus*	2
Cynosurus cristatus	2	*Lolium perenne*	1	*Cynosurus cristatus*	5	*Lolium perenne*	10
Bromus hordeaceus	5	*Cynosurus cristatus*	1	*Bromus hordeaceus*	5	*Cynosurus cristatus*	10
Poa trivialis	2	*Bromus hordeaceus*	10	*Phleum pratense*	2	*Bromus hordeaceus*	3
Briza media	1	*Poa trivialis*	2	Deschampsia cespitosa	1	Deschampsia cespitosa	5
Carex nigra	2	*Dactylis glomerata*	1	*Juncus effusus*	10	*Juncus effusus*	15
Rumex acetosa	5	*Festuca rubra*	2	*Carex nigra*	3	*Plantago lanceolata*	30
Trifolium pratense	20	*Plantago lanceolata*	40	*Plantago lanceolata*	20	*Rumex acetosa*	30
Rhinanthus minor	40	*Trifolium pratense*	4	*Rumex acetosa*	20	*Trifolium pratense*	50
Sanguisorba officinalis	2	*Rhinanthus minor*	30	*Trifolium pratense*	20	*Rhinanthus minor*	40
Bellis perennis	30	*Sanguisorba officinalis*	1	*Rhinanthus minor*	30	*Bellis perennis*	20
Filipendula ulmaria	20	*Ranunculus repens*	5	*Ranunculus repens*	10	*Filipendula ulmaria*	10
Alchemilla vulgaris agg.	25	*Bellis perennis*	40	*Bellis perennis*	30	*Myostis discolor*	2
Taraxacum officinale agg.	10	*Filipendula ulmaria*	5	*Filipendula ulmaria*	10	*Alchemilla vulgaris* agg.	1
Stellaria media	1	*Taraxacum officinale* agg.	5	*Myostis discolor*	1	*Taraxacum officinale* agg.	5
				Alchemilla vulgaris agg.	3		
				Taraxacum officinale agg.	10		
				Caltha palustris	5		

Bottom Meadow

	3		**4**		**5**		**1**
Anthoxanthum odoratum	10	*Anthoxanthum odoratum*	10	*Anthoxanthum odoratum*	5	*Anthoxanthum odoratum*	4
Lolium perenne	10	*Holcus lanatus*	1	*Bromus hordeaceus*	5	*Lolium perenne*	3
Bromus hordeaceus	10	*Lolium perenne*	1	*Poa trivialis*	2	*Cynosurus cristatus*	2

Poa trivialis	2	*Bromus hordeaceus*	5	*Dactylis glomerata*	1	*Bromus hordeaceus*	1
Dactylis glomerata	1	Deschampsia cespitosa	1	Deschampsia cespitosa	1	*Poa trivialis*	5
Plantago lanceolata	30	*Juncus effusus*	2	*Carex nigra*	20	*Plantago lanceolata*	10
Rumex acetosa	20	*Carex nigra*	30	Carex panacea	10	Rumex obtusifolius	15
Trifolium pratense	30	*Rumex acetosa*	15	*Trifolium pratense*	10	*Rumex acetosa*	1
Rhinanthus minor	60	*Trifolium pratense*	10	*Rhinanthus minor*	2	*Trifolium pratense*	40
Ranunculus repens	4	*Rhinanthus minor*	15	*Ranunculus ficaria*	5	Ranunculus bulbosus	30
Ranunculus ficaria	2	*Ranunculus repens*	1	*Bellis perennis*	40	*Ranunculus ficaria*	1
Bellis perennis	20	*Bellis perennis*	10	*Filipendula ulmaria*	5	*Anthriscus sylvestris*	1
Myostis discolor	1	*Filipendula ulmaria*	5	*Myostis discolor*	1	*Bellis perennis*	40
Cerastium fontanum	1	*Cerastium fontanum*	2	*Taraxacum officinale* agg.	20	*Cerastium fontanum*	5
Cardamine pratensis	1	*Alchemilla vulgaris* agg.	1	*Cardamine pratensis*	1	*Alchemilla vulgaris* agg.	4
		Taraxacum officinale agg.	5			*Taraxacum officinale* agg.	20
		Stellaria media	1			*Euphrasia nemorosa*	2
		Caltha palustris	2				

	2		**3**		**4**		**5**
Anthoxanthum odoratum	5	*Anthoxanthum odoratum*	10	*Anthoxanthum odoratum*	4	*Anthoxanthum odoratum*	6
Lolium perenne	2	*Alopecurus pratensis*	2	*Lolium perenne*	5	*Lolium perenne*	10
Cynosurus cristatus	1	*Lolium perenne*	5	*Cynosurus cristatus*	2	*Bromus hordeaceus*	10
Bromus hordeaceus	5	*Bromus hordeaceus*	5	*Bromus hordeaceus*	2	*Poa trivialis*	4
Poa trivialis	4	*Poa trivialis*	5	*Poa trivialis*	5	*Plantago lanceolata*	30
Plantago lanceolata	40	*Plantago lanceolata*	30	*Poa annua*	1	*Trifolium pratense*	40
Rumex acetosa	10	*Rumex acetosa*	40	Deschampsia cespitosa	1	*Geranium sylvaticum*	2
Trifolium pratense	20	*Trifolium pratense*	20	*Rumex acetosa*	30	*Ranunculus acris*	20

Geranium sylvaticum	1	*Ranunculus acris*	20	*Trifolium pratense*	40	*Ranunculus ficaria*	1
Ranunculus acris	5	Ranunculus bulbosus	5	*Ranunculus acris*	5	*Anthriscus sylvestris*	1
Ranunculus bulbosus	1	*Anthriscus sylvestris*	25	Ranunculus bulbosus	5	*Bellis perennis*	40
Anthriscus sylvestris	10	*Bellis perennis*	40	*Bellis perennis*	30	*Cerastium fontanum*	5
Bellis perennis	20	*Alchemilla vulgaris* agg.	20	*Cerastium fontanum*	2		
Cerastium fontanum	2			*Alchemilla vulgaris* agg.	20		
Taraxacum officinale agg.	20			*Taraxacum officinale* agg.	10		
Conopodium majus	1			Geum rivale	10		
Geum rivale	1						

Appendix 6. New House Farm Hay seed kg^{-1}.

	total/kg				
	1	2	3	4	5
Poa trivialis	116.5	32.8	36.2	145.3	123.6
Poa pratense	0.9	3.3	1.4	1.3	0.0
Bromus hordeaceus	17.4	*0.0*	2.9	66.7	30.6
Lolium perenne	32.1	*4.9*	2.9	77.3	40.3
Anthoxanthum odoratum	6.4	1.6	8.7	32.0	11.1
Alopecurus pratense	2.8	1.6	2.9	1.3	1.4
Phleum pratesne	1.8	1.6	0.0	0.0	0.0
Cynosurus cristatus	1.8	0.0	0.0	1.3	2.8
Agrostis capilaris	0.9	1.6	0.0	0.0	0.0
Deschampsia ceaspitosa	0.0	0.0	5.8	*0.0*	0.0
Festuca pratense	0.0	0.0	1.4	*0.0*	0.0
Festuca rubra	0.0	0.0	0.0	1.3	1.4
Holcus lanatus	0.0	0.0	0.0	1.3	2.8
Helictotrichon	0.0	0.0	0.0	1.3	0.0

pubescens					
Juncus bufonius	0.0	*1.6*	0.0	0.0	0.0
Juncus effusus	0.0	*1.6*	2.9	*0.0*	0.0
Rumex acetosa	0.9	*1.6*	0.0	*1.3*	1.4
Bellis perennis	78.0	*32.8*	23.2	*274.7*	62.5
Plantago lanceolata	118.3	*37.7*	11.6	*202.7*	91.7
Trifolium sp.	11.0	*3.3*	1.4	*1.3*	8.3
Myostis sp.	7.3	*8.2*	0.0	*24.0*	13.9
Ranunculus sp	0.9	*0.0*	1.4	*1.3*	1.4
Cerastium fontanum	0.9	0.0	1.4	2.7	0.0
Chamerion angustifolium	0.9	0.0	0.0	0.0	0.0
Conopodium majus	0.0	1.6	0.0	1.3	0.0
Cirsium arvense	0.0	1.6	0.0	0.0	0.0
Polygonum bistorta	0.0	0.0	0.0	1.3	0.0

Appendix 7 New House Farm manure seed kg^{-1}.

Fresh				*3 month old*			
	A		B		A		B
Poa trivialis	20.5	*Poa trivialis*	7.1	*Poa trivialis*	11.36364	*Poa trivialis*	13.95349
Plantago lanceolata	2.6	*Juncus bufonius*	1.4	*Anthoxanthum odoratum*	3.409091	*Anthoxanthum odoratum*	4.651163
Juncus bufonius	2.6	*Lolium perenne*	1.4	*Myosotis sp*	3.409091	*Plantago lanceolata*	2.325581
Lolium perenne	2.6			*Bellis pernenis*	2.272727	*Juncus bufonius*	1.162791
Myostis sp.	1.3			*Rumex acetosa*	2.272727		
Anthxanthum odoratum	1.3			*Urtica dioica*	1.136364		

6 month old			
	A		
Poa triv	3.125	*Poa triv*	5.102041
		J. effusus	2.040816

Appendix 8. New House Farm dung seed kg^{-1}.

	NH1A		NH1B
Poa trivialis	8.0	Poa trivialis	5.1
Juncus effusus	6.7	Juncus effusus	7.6
Trifolium pratense	1.3	Juncus bufonius	15.2
Poa pratensis	1.3	Lolium perenne	1.3
Cynosurus cristatus	1.3	Trifolium pratense	6.3
		Poa pratensis	5.1
		Bromus hordeaceus	3.8
		Cardamine pratense	1.3

	NH2A		NH2B
Poa trivialis	17.1	Poa trivialis	17.6
Plantago laceolata	3.7	Juncus effusus	4.1
Juncus bufonius	6.1	Cynosurus cristatus	1.4
Bellis perennis	7.3		

Lolium perenne	2.4		

	NH3A		NH3B
Poa trivialis	18.6	Poa trivialis	10.1
Plantago laceolata	10.5	Plantago laceolata	13.9
Juncus effusus	5.8	Bellis perennis	2.5
Cynosurus cristatus	1.2	Lolium perenne	5.1
		Myosotis discolor	3.8

Appendix 9. List of NVC sub-communities used in the PCA analysis.

MG 7	*Lolium perenne* leys and related grassland.
MG 7A	*Lolium perenne-Trifolium repens* leys
MG 7B	*Lolium perenne Poa trivialis* leys
MG 7C	*Lolium perenne-Alopecurus pratensis-Festuca pratensis* grassland
MG 7D	*Lolium perenne-Alopecurus pratensis* grassland
MG 7E	*Lolium perenne-Plantago lanceolata* grassland
MG 7F	*Lolium perenne-Poa pratensis* grassland
MG 6	*Lolium perenne-Cynosurus cristatus* grassland
MG 6A	*Lolio-Cynosuretum typicum*
MG 6B	*Anthoxanthum odoratum* sub-community
MG 6C	*Trisetum flavescens* sub-community
MG 5	*Cynosurus cristatus-Centaurea nigra* grassland
MG 5A	*Lathyrus pratensis* sub-community
MG 5B	*Galium verum* sub-community
MG 3	*Anthoxanthum odoratum-Geranium sylvaticum* grassland.
MG 3A	*Bromus hordeaceus* sub-community
MG 3B	*Briza media* sub-community

Appendix 10. List of remaining species with viable seed in soil samples taken from Piper Hole.

Dactylis glomerata	14.3	0.1	0.0	0.0	1.47	0.165
Cynosurus cristatus	14.3	0.2	7.1	0.1	0.81	0.435
Urtica dioica	14.3	0.2	14.3	0.1	0.56	0.583
Chamerion angustifolium	14.3	0.1	21.4	0.2	-0.56	0.883
Veronica chamaedrys	14.3	0.3	7.1	0.1	1.00	0.336
Tarxacum officinale agg.	14.3	0.6	0.0	0.0	1.38	0.189
Arrehenatharu m elatius	7.1	0.1	0.0	0.0	1.00	0.336
Agrostis stonlonifera	7.1	0.1	0.0	0.0	1.00	0.336
Rumex obtusifolius	7.1	0.1	14.3	0.1	-0.56	0.584
Cirsium helenioides	7.1	0.1	7.1	0.1	0.0	1.000
Festuca pratensis	0.0	0.0	7.1	0.1	-1.00	0.336
Digitalis purpurea	0.0	0.0	7.1	0.1	-1.00	0.336

Appendix 11. List of remaining species with viable seed in soil samples taken from New House Farm.

Deschampsia cespitosa	25.00	0.833	25	0.8333	0.97	0.352
Lolium perenne	16.67	0.250	0	0.00	1.39	0.191
Agrostis capillaris	16.67	0.167	16.67	0.167	0.00	1.000
Poa annua	16.67	0.167	0	0.00	1.48	0.166
Phleum pratensis	16.67	1.446	0	0.00	1.20	0.256
Cynosurus cristatus	16.67	0.250	0	0.00	1.39	0.191
Polygonum bistorta	16.67	0.167	8.33	0.083	0.56	0.586
Briza media	8.33	0.0833	0.00	0.000	1.00	0.339
Dactylis glomerata	8.33	0.0833	8.33	0.0833	0.00	1.000
Chamerion angustifolium	8.33	0.083	16.67	0.167	-1.00	0.339
Conopodium majus	8.33	0.0833	8.33	0.0833	0.00	1.000
Rhinanathus minor	8.33	0.50	0.00	0.00	1.00	0.339
Prunella vulgaris	8.33	0.0833	0	0.00	1.00	0.339
Anthriscus sylvestris	8.33	0.0833	0	0.00	1.00	0.339
Stellaria media	8.33	0.0909	0.00	0.000	1.00	0.341

Festuca pratensis	0.00	0.00	8.33	0.083	-1.00	0.339
Rumex obtusifolius	0.00	0.000	8.33	0.0833	-1.00	0.339
Plantago major	0.00	0.000	8.33	0.0833	-1.00	0.339
Spergula arvenis	0.00	0.00	8.33	0.0833	-1.00	0.000
Senecio jacobea	0.00	0.00	8.33	0.0833	-1.00	0.000
Urtica dioica	0.00	0.00	8.33	0.0833	-1.00	0.000

Appendix 12. List of species percentage cover for each plot within the Piper Hole disturbance experiment.

Control 1a	2000	2002
Bromus hordeaceus	2	1
Poa trivialis	5	4
Anthoxanthum odoratum	2	3
Holcus lanatus	1	1
Rumex acetosa	5	2
Anthriscus sylvestris	1	1
Alchemilla vulgaris agg.	3	2
Ranunculus acris	10	20
Trifolium pratense	15	5
Cerastium fontanum	0	1
Rhinanthus minor	2	1
Galium saxatile	1	0
Vicia sepium	5	1
Lathyrus pratensis	0	0
Filipendula ulmaria	5	1
Bare Ground	0	10

Control 1b	2000	2002
Bromus hordeaceus	1	2
Lolium perenne	3	2
Poa trivialis	5	4
Anthoxanthum odoratum	1	0
Holcus lanatus	3	2
Rumex acetosa	15	10
Myostis discolor	1	1
Anthriscus sylvestris	1	2
Ranunculus acris	10	10
Trifolium pratense	25	20
Sanguisorba officinalis	10	5
Galium saxatile	1	0
Stellaria media	1	2
Filipendula ulmaria	10	10
Bare Ground	0	10

Control 1c	2000	2002
Bromus hordeaceus	1	0
Lolium perenne	1	0
Poa trivialis	7	5
Anthoxanthum odoratum	1	5
Alopecurus pratensis	1	1
Holcus lanatus	1	2
Anthriscus sylvestris	1	1
Ranunculus acris	10	10
Trifolium pratense	30	20
Cerastium fontanum	1	0
Rhinanthus minor	1	0
Stellaria media	1	2
Vicia sepium	1	1
Filipendula ulmaria	10	5

CONT1c	2000	2002
Bromus hordeaceus	1	1
Lolium perenne	10	5
Poa trivialis	8	5
Anthoxanthum odoratum	1	1
Phleum pratense	3	2
Rumex acetosa	15	20
Anthriscus sylvestris	0	1
Ranunculus acris	25	30
Trifolium pratense	25	30
Cerastium fontanum	0	1
Rhinanthus minor	3	1
Stellaria media	2	2
Vicia sepium	0	1
Filipendula ulmaria	10	5

Control 2a	2000 C	2002
Bromus hordeaceus	3	2
Lolium perenne	3	2

Control 2b	2000	2002
Bromus hordeaceus	5	10
Lolium perenne	5	10

Poa trivialis	3	4
Anthoxanthum odoratum	3	2
Dactylis glomerata	0	2
Alopecurus pratensis	5	5
Agrostis capillaris	1	0
Plantago lanceolata	1	5
Rumex obtusifolius	0	0
Rumex acetosa	20	10
Myostis discolor	2	1
Conopodium majus	0	0
Anthriscus sylvestris	0	2
Alchemilla vulgaris agg.	1	1
Geranium sylvaticum	2	1
Ranunculus acris	25	20
Trifolium pratense	30	20
Sanguisorba officinalis	1	2

Poa trivialis	5	10
Anthoxanthum odoratum	1	0
Dactylis glomerata	3	0
Alopecurus pratensis	1	10
Rumex acetosa	25	30
Myostis discolor	2	2
Anthriscus sylvestris	0	20
Alchemilla vulgaris agg.	0	10
Ranunculus acris	30	30
Trifolium pratense	20	40
Cerastium fontanum	2	2
Bellis perennis	1	0
Taraxacum officinale agg.	1	0

Control 2c	2000	2002
Bromus hordeaceus	2	10
Lolium perenne	0	10
Poa trivialis	2	10
Anthoxanthum odoratum	1	0
Alopecurus pratensis	2	10
Rumex acetosa	10	30
Myostis discolor	1	2
Anthriscus sylvestris	1	20
Alchemilla vulgaris agg.	1	10
Ranunculus acris	25	30
Trifolium pratense	25	40
Cerastium fontanum	1	2
Taraxacum officinale agg.	1	0

Control 2d	2000	2002
Lolium perenne	10	10
Poa trivialis	10	30
Anthoxanthum odoratum	5	5
Alopecurus pratensis	20	5
Rumex acetosa	10	10
Anthriscus sylvestris	0	10

Turf Removal 1a	2000	2002
Bromus hordeaceus	1	0
Lolium perenne	2	5
Poa trivialis	1	20
Anthoxanthum odoratum	1	15
Alopecurus pratensis	0	15

Alchemilla vulgaris agg.	1	0
Ranunculus acris	10	20
Trifolium pratense	30	30
Bellis perennis	1	0
Taraxacum officinale agg.	1	1
Cirsium arvense	1	0
Rhinanthus minor	0	1

Holcus lanatus	1	0
Rumex acetosa	5	10
Myostis discolor	0	1
Ranunculus acris	2	3
Trifolium pratense	5	10
Sanguisorba officinalis	0	5
Cerastium fontanum	0	1
Bellis perennis	1	0
Bare Ground	60	0

Turf removal 1b	2000	2002
Bromus hordeaceus	0	2
Lolium perenne	3	1
Poa trivialis	2	4
Anthoxanthum odoratum	2	2
Alopecurus pratensis	0	2
Rumex acetosa	10	4
Anthriscus sylvestris	1	5
Ranunculus acris	2	5
Trifolium pratense	5	0
Sanguisorba officinalis	0	5
Stellaria media	1	0
Filipendula ulmaria	0	5
Bare Ground	40	0

Turf Removal 1c	2000	2002
Bromus hordeaceus	1	1
Lolium perenne	1	5
Poa trivialis	2	4
Anthoxanthum odoratum	5	0
Holcus lanatus	1	0
Plantago lanceolata	0	2
Rumex acetosa	5	10
Conopodium majus	1	0
Anthriscus sylvestris	0	10
Ranunculus acris	5	5
Trifolium pratense	10	10
Sanguisorba officinalis	0	1
Cerastium fontanum	1	5
Stellaria media	1	0
Vicia sepium	0	1
Lathyrus pratensis	2	0
Filipendula ulmaria	0	2
Bare Ground	50	0

Turf Removal 1d	2000	2002
Bromus hordeaceus	2	0
Lolium perenne	1	0
Poa trivialis	2	40
Alopecurus pratensis	0	5
Holcus lanatus	1	2
Plantago lanceolata	1	0
Rumex acetosa	5	20
Ranunculus acris	5	1
Trifolium pratense	10	20
Cerastium fontanum	1	0
Stellaria media	0	1
Filipendula ulmaria	0	1
Bare Ground	65	0

Turf Removal 2a	2000	2002
Bromus hordeaceus	1	2
Lolium perenne	1	2
Poa trivialis	1	10
Anthoxanthum odoratum	1	0
Phleum pratense	1	0
Plantago lanceolata	1	20
Rumex acetosa	2	20
Anthriscus sylvestris	1	1
Ranunculus acris	1	20
Sanguisorba officinalis	1	1
Cerastium fontanum	0	2
Bellis perennis	1	0
Rhinanthus minor	0	1

Stellaria media	0	1
Bare Ground	55	0

Turf Remval 2b	2000	2002
Bromus hordeaceus	1	2
Lolium perenne	2	0
Poa trivialis	2	10
Poa annua	1	0
Anthoxanthum odoratum	1	0
Dactylis glomerata	2	5
Alopecurus pratensis	0	5
Holcus lanatus	1	0
Phleum pratense	1	0
Plantago lanceolata	0	10
Rumex acetosa	1	10
Anthriscus sylvestris	1	1
Alchemilla vulgaris agg.	0	10
Ranunculus acris	1	20
Sanguisorba officinalis	0	25
Cerastium fontanum	1	1
Cirsium arvense	1	0
Rhinanthus minor	0	1
Bare Ground	30	0

Turf Removal 2c	2000	2002
Bromus hordeaceus	0	20
Lolium perenne	2	20
Poa trivialis	2	0
Anthoxanthum odoratum	0	5
Dactylis glomerata	1	2
Alopecurus pratensis	0	20
Agrostis capillaris	2	0
Plantago lanceolata	0	5
Rumex acetosa	2	15
Myostis discolor	2	1
Conopodium majus	2	0
Anthriscus sylvestris	0	5
Geranium sylvaticum	1	0
Ranunculus repens	0	0
Ranunculus acris	2	10
Trifolium pratense	0	30
Sanguisorba officinalis	1	0
Cerastium fontanum	1	2
Taraxacum officinale agg.	1	10
Bare Ground	40	0

Turf Removal 2d	2000	2002
Bromus hordeaceus	1	20
Lolium perenne	1	1
Poa trivialis	2	0
Poa annua	1	0
Anthoxanthum odoratum	2	5
Dactylis glomerata	0	5
Alopecurus pratensis	0	20
Agrostis capillaris	2	0
Rumex acetosa	0	20
Myostis discolor	1	0
Conopodium majus	1	0
Anthriscus sylvestris	0	40
Alchemilla vulgaris agg.	1	0
Ranunculus acris	2	10
Trifolium pratense	0	40
Cerastium fontanum	0	1
Bellis perennis	1	0

Manure Substitute 1a	2000	2002
Bromus hordeaceus	0	2
Lolium perenne	2	5
Poa trivialis	2	2
Holcus lanatus	0	3
Rumex acetosa	5	10
Anthriscus sylvestris	2	10
Ranunculus acris	5	20
Trifolium pratense	15	20
Stellaria media	2	5
Vicia sepium	0	1
Filipendula ulmaria	0	5
Bare Ground	20	5

Galium saxatile ?	0	2
Bare Ground	30	0

Manure Substitute 1b	2000	2002
Bromus hordeaceus	3	5
Lolium perenne	3	5
Poa trivialis	5	5
Anthoxanthum odoratum	0	5
Dactylis glomerata	0	1
Alopecurus pratensis	1	2
Holcus lanatus	0	1
Rumex acetosa	10	10
Ranunculus acris	5	10
Trifolium pratense	10	20
Sanguisorba officinalis	1	0
Cerastium fontanum	0	4
Rhinanthus minor	1	0
Filipendula ulmaria	1	1
Bare Ground	30	0

Manure Substitute 1c	2000	2002
Bromus hordeaceus	1	3
Lolium perenne	2	5
Poa trivialis	1	4
Anthoxanthum odoratum	0	3
Holcus lanatus	1	2
Rumex acetosa	10	20
Ranunculus acris	20	30
Trifolium pratense	10	20
Cerastium fontanum	1	1
Taraxacum officinale agg.	1	0
Rhinanthus minor	1	0
Stellaria media	2	0
Filipendula ulmaria	1	1
Bare Ground	10	0

Manure Substitute 1d	2000	2002
Bromus hordeaceus	2	1
Lolium perenne	5	10
Poa trivialis	5	5
Alopecurus pratensis	0	1
Holcus lanatus	1	0
Rumex acetosa	25	30
Myostis discolor	0	1
Anthriscus sylvestris	1	0
Ranunculus acris	0	1
Trifolium pratense	20	30
Sanguisorba officinalis	0	5
Cerastium fontanum	1	0
Rhinanthus minor	1	1
Stellaria media	1	2
Bare Ground	5	10

Manure Substitute 2a	2000	2002
Bromus hordeaceus	2	5
Lolium perenne	2	2
Poa trivialis	2	5
Anthoxanthum odoratum	0	5
Alopecurus pratensis	0	5
Holcus lanatus	2	0
Phleum pratense	2	0
Plantago lanceolata	2	0
Rumex acetosa	0	20
Myostis discolor	2	0
Alchemilla vulgaris agg.	0	10
Ranunculus repens	1	0
Ranunculus acris	0	30
Ranunculus bulbosus	1	0
Trifolium repens	0	0
Trifolium pratense	2	30
Sanguisorba officinalis	1	5
Cerastium fontanum	2	2
Rhinanthus minor	0	2
Bare Ground	20	0

Manure Substitute 2b	2000	2002
Lolium perenne	2	2

Manure Substitute 2c	2000	2002
Bromus hordeaceus	1	0

Poa trivialis	5	10
Poa annua	1	0
Anthoxanthum odoratum	2	0
Alopecurus pratensis	1	2
Holcus lanatus	2	4
Plantago lanceolata	5	0
Rumex obtusifolius	0	10
Myostis discolor	2	10
Alchemilla vulgaris agg.	1	2
Geranium sylvaticum	0	10
Ranunculus repens	2	0
Ranunculus bulbosus	0	5
Trifolium pratense	2	0
Sanguisorba officinalis	10	40
Cerastium fontanum	2	0
Bellis perennis	1	2
Galium saxatile	0	1
Bare Ground	25	0

Lolium perenne	2	0
Poa trivialis	2	0
Poa annua	1	5
Anthoxanthum odoratum	2	0
Dactylis glomerata	0	5
Holcus lanatus	0	5
Phleum pratense	2	0
Rumex obtusifolius	0	20
Rumex acetosa	1	0
Myostis discolor	2	30
Anthriscus sylvestris	1	0
Alchemilla vulgaris agg.	0	20
Ranunculus repens	1	0
Ranunculus bulbosus	2	30
Trifolium pratense	2	0
Sanguisorba officinalis	0	30
Bellis perennis	0	3
Taraxacum officinale agg.	1	0
Galium saxatile	0	1
Stellaria media	0	1

Manure Substitute 2d	2000	2002
Bromus hordeaceus	2	0
Lolium perenne	0	10
Poa trivialis	2	0
Poa annua	1	30
Anthoxanthum odoratum	2	0
Dactylis glomerata	0	10
Alopecurus pratensis	2	10
Rumex obtusifolius	0	20
Rumex acetosa	1	0
Myostis discolor	0	10
Conopodium majus	1	1
Alchemilla vulgaris agg.	0	30
Geranium sylvaticum	10	10
Ranunculus repens	0	2
Sanguisorba officinalis	0	30
Bellis perennis	1	2
Taraxacum officinale agg.	2	0
Galium saxatile	0	2

FYM 1a	2000	2002
Bromus hordeaceus	2	0
Lolium perenne	5	2
Poa trivialis	1	3
Anthoxanthum odoratum	1	5
Dactylis glomerata	1	0
Alopecurus pratensis	1	3
Holcus lanatus	1	1
Plantago lanceolata	0	2
Rumex acetosa	5	10
Anthriscus sylvestris	1	1
Alchemilla vulgaris agg.	0	5
Ranunculus acris	5	5
Trifolium pratense	5	15
Sanguisorba officinalis	1	3
Cerastium fontanum	1	1
Stellaria media	1	0
Vicia sepium	1	3
Lathyrus pratensis	1	0

Filipendula ulmaria	0	10
Bare Ground	20	0

FYM 1b	2000	2002
Bromus hordeaceus	2	4
Lolium perenne	2	0
Poa trivialis	0	5
Anthoxanthum odoratum	2	0
Dactylis glomerata	1	5
Plantago lanceolata	5	3
Rumex acetosa	10	10
Anthriscus sylvestris	0	2
Ranunculus acris	0	10
Trifolium pratense	20	20
Sanguisorba officinalis	0	2
Cerastium fontanum	1	2
Stellaria media	1	0
Vicia sepium	1	0
Filipendula ulmaria	0	2
Bare Ground	30	0

FYM 1c	2000	2002
Bromus hordeaceus	2	3
Lolium perenne	1	1
Poa trivialis	2	4
Anthoxanthum odoratum	1	0
Alopecurus pratensis	0	1
Holcus lanatus	1	1
Agrostis capillaris	2	0
Plantago lanceolata	1	0
Rumex acetosa	10	20
Anthriscus sylvestris	0	1
Ranunculus acris	15	5
Trifolium pratense	10	20
Cerastium fontanum	1	0
Rhinanthus minor	1	0
Galium saxatile ?	1	0
Stellaria media	0	2
Lathyrus pratensis	1	1
Filipendula ulmaria	10	20
Bare Ground	30	0

FYM 1d	2000	2002
Lolium perenne	5	2
Poa trivialis	2	15
Anthoxanthum odoratum	1	0
Alopecurus pratensis	0	5
Holcus lanatus	4	0
Phleum pratense	1	0
Agrostis capillaris	1	0
Plantago lanceolata	3	4
Rumex acetosa	5	10
Anthriscus sylvestris	1	0
Ranunculus acris	5	15
Trifolium pratense	10	30
Sanguisorba officinalis	3	20
Cerastium fontanum	1	0
Rhinanthus minor	1	1
Galium saxatile	0	0
Stellaria media	1	2
Filipendula ulmaria	5	15
Bare Ground	25	0

FYM 2a	2000	2002
Bromus hordeaceus	1	5
Lolium perenne	2	20
Anthoxanthum odoratum	1	0
Dactylis glomerata	1	1
Agrostis capillaris	1	0
Rumex acetosa	1	20
Myostis discolor	1	3
Ranunculus repens	2	0
Ranunculus acris	0	30
Sanguisorba officinalis	1	0
Cerastium fontanum	1	2
Bellis perennis	1	0
Taraxacum officinale agg.	0	10
Bare Ground	10	0

FYM 2b	2000	2002
Bromus hordeaceus	0	5
Lolium perenne	1	15
Poa trivialis	0	0
Poa annua	0	5
Anthoxanthum odoratum	0	5
Dactylis glomerata	0	10
Alopecurus pratensis	2	0
Phleum pratense	1	0
Agrostis capillaris	1	0
Rumex acetosa	1	20
Myostis discolor	0	2
Anthriscus sylvestris	0	1
Ranunculus repens	1	0
Ranunculus acris	0	20
Trifolium repens	1	0
Trifolium pratense	2	30
Sanguisorba officinalis	1	30
Cerastium fontanum	1	2
Bellis perennis	1	0
Taraxacum officinale agg.	1	0
Bare Ground	10	0

FYM 2c	2000	2002
Bromus hordeaceus	2	2
Lolium perenne	2	10
Poa annua	2	5
Anthoxanthum odoratum	1	2
Dactylis glomerata	2	0
Alopecurus pratensis	1	0
Agrostis capillaris	1	0
Rumex acetosa	1	5
Myostis discolor	1	0
Anthriscus sylvestris	0	1
Geranium sylvaticum	1	60
Ranunculus repens	2	0
Ranunculus acris	0	5
Trifolium pratense	1	40
Sanguisorba officinalis	1	40
Cerastium fontanum	1	1
Rhinanthus minor	0	1
Bare Ground	30	0

FYM 2d	2000	2002
Bromus hordeaceus	1	1
Lolium perenne	2	20
Poa trivialis	1	0
Poa annua	2	5
Dactylis glomerata	1	5
Phleum pratense	1	0
Plantago lanceolata	0	20
Rumex acetosa	1	10
Myostis discolor	1	0
Conopodium majus	1	0
Anthriscus sylvestris	1	5
Geranium sylvaticum	0	30
Ranunculus acris	0	5
Trifolium pratense	1	5
Sanguisorba officinalis	0	1
Cerastium fontanum	1	2
Bellis perennis	1	0
Bare Ground	30	0