Journal of Ecology
British Ecological Society
Journal of Ecology 2008, **96**, 894–902 doi: 10.1111/j.1365-2745.2008.01403.x

Sustaining ecosystem services in ancient limestone grassland: importance of major component plants and community composition

Gareth K. Phoenix[1]*, David Johnson[2], J. Philip Grime[1] and Rosemary E. Booth[1]

[1]*Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK; and* [2]*School of Biological Sciences, University of Aberdeen, Cruickshank Building, St Machar Drive, Aberdeen AB24 3UU, UK*

Summary

1. Limestone grasslands are among the most floristically rich ecosystems in northern Europe and have recently been shown to sustain ecosystem functioning under conditions of heavy nitrogen (N) deposition. Here we report a long-term mesocosm experiment, designed to measure the importance of major component plants and community composition in providing ecosystem services (in particular preventing leaching of inorganic N).

2. A number of important steps were taken in the experimental design to increase conformity of the experimental communities to real limestone pasture, including using natural soils, plant cuttings of limestone grassland provenance and simulated management. Furthermore, communities were allowed to establish for 8 years before measurements were made.

3. Leaching of NO_3^- and NH_4^+ were found to differ by up to a considerable two orders of magnitude according to functional group composition, with sedges and forbs leaching the most. Grass communities and communities containing all three functional groups (grasses, sedges and forbs) leached similarly low amounts of NO_3^- and NH_4^+.

4. Levels of soil NO_3^- and NH_4^+ followed a similar pattern of inter-community differences to leaching, while vegetation biomass and vegetation N stocks were very similar between communities consisting of only grasses, only sedges and the most species rich community (4 grasses + 4 sedges + 4 forbs).

5. Perennial grasses appeared to play the key role in inorganic N retention, with communities consisting of these plants having equally low leaching as more species rich communities. Indeed, most ecosystem services were performed comparatively well by grass communities as by the most species rich community.

6. No overall effects on provision of services resulted from mixing functional groups or increasing the number of species representing each functional group.

7. *Synthesis*. This work shows that more than order-of-magnitude differences in provision of ecosystem services can occur between species that coexist naturally in ancient limestone pasture. However, despite these large differences, because of the key role played by grasses, expected shifts in community composition anticipated as a result of N pollution may not impair the capacity of these systems to provide ecosystems services.

Key-words: biodiversity, calcareous grassland, community composition, ecosystem function, ecosystem services, multifunctionality, nitrogen deposition, nitrogen leaching, plant functional types, seasonal dynamics

Introduction

Calcareous grasslands are among the most floristically rich plant communities of northern Europe and have considerable conservation and amenity value. The floristic diversity, however, is threatened by atmospheric nitrogen (N) deposition and agricultural improvement (Bobbink *et al.* 1998; Preston *et al.* 2002; Bennie *et al.* 2006). Among these grasslands are the species rich ancient limestone grasslands of the Peak District National Park (northern England) that for many decades have been subjected to increased rates of atmospheric N deposition. This has raised major concern that plant

*Correspondence author. E-mail: g.phoenix@sheffield.ac.uk

species richness may decline and these ecosystems may lose their capacity to store N and to prevent its leakage into groundwater. To a surprising extent such fears have not been confirmed. In long-term plot experiments (at Wardlow Hay Cop, National Nature Reserve, Cressbrookdale, UK) involving experimental additions of N, the limestone grassland has shown considerable capacity to accumulate much of the N inputs, providing the important ecosystem service of clean groundwater provision even in the face of chronic N pollution (Phoenix *et al.* 2003, 2004). However, the importance of the component plants, plant community composition and species diversity in driving that continuation of ecosystem service remains unknown and it is unknown whether potential future shifts in the plant community threaten that continuation of service.

There is now a large body of work investigating and debating the possible consequences of biodiversity loss for the functioning of ecosystems (see Hooper *et al.* 2005 for review). Many studies using experimental herbaceous plant communities have reported a loss of functioning with declining species and functional group richness (such as loss of productivity and soil nutrient status (Naeem *et al.* 1994, 1995; Hooper & Vitousek 1997; Tilman *et al.* 1997; Hector *et al.* 1999; Lanta & Leps 2006), increased N leaching (Scherer-Lorenzen *et al.* 2003) and reduced stability or resistance to invasion (Symstad 2000; Tilman *et al.* 2006)). While debate remains on the interpretation of some findings (see for example Thompson *et al.* 2005 and Hector *et al.* 2007) all studies agree that it is community composition (i.e. the identity of the plants forming the community) that has the greatest control on ecosystem function (compared to species or functional group richness). In limestone grassland, therefore, particular plant species or groups of species may provide the fundamental role in sustaining ecosystem services under pressures from N pollution.

In general, most previous plant diversity–ecosystem function studies have used the research approach of constructing from seed, model herbaceous plant communities with controlled species and functional group richness (e.g. Tilman *et al.* 1996; Hector *et al.* 1999; Mulder *et al.* 2002; Scherer-Lorenzen *et al.* 2003). In order to contribute to this type of fundamental research it is not always essential that the synthesized communities conform in detail with a single specific ecosystem. Indeed, an element of generality to the design can be desirable in such research which aims to make observations that may be broadly applicable. Where, however, research aims to understand the consequences of community change in a particular ecosystem, it is important that experiments have close approximation to the conditions prevailing in the particular ecosystem under study (Grime 2001, 2002). Furthermore, when investigating a specific ecosystem, the main threats posed to biodiversity may be known and the likely shifts in vegetation resulting from those pressures may also be understood. This allows the luxury of placing greater emphasis on comparing the capacity of particular groups of plants (likely to be lost or gained under environmental change) in performing ecosystem services.

This paper presents the results of an experiment designed to identify the role of major component plants and community composition upon the capacity of ancient limestone grassland to perform ecosystem services (in particular the retention of N and maintaining low inorganic N loads in leachate). Specifically it addresses the following questions: (i) how, and to what extent, do communities constructed of major plant functional groups within limestone grassland (grasses, sedges and forbs) differ in their ability to provide ecosystem services; (ii) do any communities differ in the provision of ecosystem services compared to the most species rich community; (iii) is there an enhanced provision of ecosystem services where communities are constructed of mixed functional groups compared to single functional groups; and (iv) are ecosystem services promoted when each functional group is represented by several species?

For this, it was necessary to create a substantial number of model communities of limestone grassland vegetation, each with controlled species composition and close similarity to the soil and management conditions typical of this type of grassland. In brief, the main essential features were: (i) the model communities were constructed from plant species all of common occurrence in, and representing major components of, limestone grassland. (ii) These species represented three dominant plant functional groups of limestone grassland; grasses, sedges and forbs. (iii) Each synthesized community was replicated four times to allow direct comparison between each using conventional statistical analysis. (iv) Communities were constructed on freshly-collected, homogenized rendzina soil removed from a limestone grassland in the vicinity of the experimental sight where the 'N retention' service had been determined previously (Phoenix *et al.* 2003). (v) To ensure limestone grassland provenance of the plants, communities were constructed using mature vegetative transplants removed from the same small area of grassland as the soil. In order to further conform to the limestone grassland turf structure, a standardised inoculum of bryophytes (from the limestone grassland) was added to each model community. (vi) Throughout the experiment the impacts of sheep were simulated by spring and late summer cutting and a simulated 'trampling' event each autumn. (vii) To allow the synthesized communities and their associated N dynamics to equilibrate, and the normal processes of competition to establish, measurements reported here were delayed until the eighth year of the experiment – making this one of the longest running studies of its type.

By placing considerable emphasis on conformity to real limestone pasture, its soils, plants and management practises, this study allows the first assessment of the importance of major plant components and community composition in providing ecosystem services in one of the most species rich ecosystems in Europe, a system of high conservation value known to be currently threatened by N deposition. By using replicated communities of some of the dominant plants in these systems we are able to directly compare their capacity to drive ecosystem function and so begin to elucidate the likely consequences of community change resulting from N deposition impacts.

Table 1. Composition of the eight experimental communities consisting of either one or three plant functional groups [(g) grass; (s) sedge; (f) forb], and one or four species representing each functional group. Each community is replicated four times

Community composition (species with functional group in italics)	Short name on figures	Functional groups in community	Functional group richness (*FGR*)	Number of species for each functional group (*Sp/FG*)
Festuca ovina (g)	1-Grass	Grass	1	1
Carex flacca (s)	1-Sedge	Sedge	1	1
Leontodon hispidus (f)	1-Forb	Forb	1	1
Festuca ovina (g) *Carex flacca* (s) *Leontodon hispidus*	1F + 1S + 1G	Grass + sedge + forb	3	1
Festuca ovina (g) *Koeleria macrantha* (g) *Helictotrichon pratense* (g) *Briza media* (g)	4-Grasses	Grass	1	4
Carex flacca (s) *C. panicea* (s) *C. caryophyllea* (s) *C. pulicaris* (s)	4-Sedges	Sedge	1	4
L. hispidus (f) *Succisa pratensis* (f) *Campanula rotundifolia* (f) *Viola riviniana* (f)	4-Forbs	Forb	1	4
Festuca ovina (g) *Koeleria macrantha* (g) *Helictotrichon pratense* (g) *Briza media* (g) *Carex flacca* (s) *C. panicea* (s) *C. caryophyllea* (s) *C. pulicaris* (s) *Leontodon hispidus* (f) *Succisa pratensis* (f) *Campanula rotundifolia* (f) *Viola riviniana* (f)	4F + 4S + 4G	Grass + sedge + forb	3	4

Methods

MESOCOSM COMMUNITIES

In 1995, model communities of limestone grassland were constructed within propylene boxes (60 × 60 × 15 cm deep) filled with homogenized natural rendzina soil removed from ancient limestone grassland at Cressbrookdale, in the Peak National Park to the south of Sheffield, UK. The hillside where N retention by limestone grassland was determined – Warlow Hay Cop – sits beside Cressbrookdale (Phoenix *et al*. 2003). Atmospheric N deposition at both the Cressbookdale field site and the experimental communities is currently *c.* 2–2.5 g N m^{-2} $year^{-1}$ (total wet plus dry deposition; Phoenix *et al*. 2003) which is typical for large areas of the Peak National Park. The depth of the soil used in the microcosms is representative of the total soil depth to limestone bedrock of the real grassland and ensured the component plants could establish natural rooting depths and structure. The calcareous soils used for this study are of approximately pH 6.8, total soil N of *c.* 10 mg g^{-1}, organic matter by loss-on-ignition *c.* 32%, and with extractable inorganic N and P of 5–40 and 3–8 μg g^{-1} soil, respectively (range results from seasonal variation).

Communities were planted with mature plant cuttings, again removed from local limestone grassland, with each community consisting of 196 individuals allocated to random locations on a uniform grid (of 14 × 14 squares) within each mesocosm box (plant spacing *c.* 4 cm). A mix of bryophytes collected from Cressbrookdale was added to the soil surface.

Eight different communities were constructed of major plant species found within the limestone grassland. These plant species represented the three main functional groups within this system – grasses, sedges or forbs (Table 1). The grasses are tussock species capable of early growth, the sedges are rhizomatous, have delayed leaf growth, are non-mycorrhizal and produce dauciform roots (Da Vies *et al*. 1973; Harley & Harley 1987), while the forbs are a more heterogeneous group in which each attains a well-defined peak in biomass in summer.

To determine the importance of multiple functional groups in ecosystem function, communities were either constructed of a single functional group or a mix of all three. To determine whether ecosystem function improved with multiple species representing each functional group, each functional group was also either represented by a single species or by four species. This experimental design therefore produces biodiversity levels in a two-way factorial design with each community consisting of either one or three plant functional groups or one or four species representing each functional group. The biodiversity treatments are therefore ‘Functional Group Richness’ (*FGR*) and ‘Species per Functional Group’ (*Sp/FG*). Each community was replicated four times.

Since establishment, communities have been maintained by twice yearly cutting (to 2.5 cm above soil), simulated sheep trampling, and regular weeding. Invaders from seed rain and seed banks were removed by hand throughout the experiment.

LEACHATE AND SOIL EXTRACTABLE NITROGEN

Mesocosms were freely draining through perforated bases covered with a layer of voile mesh to stop loss of particulate matter. Leachate was trapped continuously for 1 year from June 2003 when the experimental communities were in their ninth year.

Leachate was collected into blacked-out 25 L containers containing thymol preservative (Hadi & Cape 1995) with sample collections taken monthly when rainfall permitted; some periods of low rainfall dictated that eight collections were made for the 12-month period of June 2003–2004. At each collection, total volume leached was recorded and leachate sub-samples were then stored frozen (–10 °C) prior to analysis. All samples were analysed colorimetrically for NO_3^- and NH_4^+ using an automated flow-injection nutrient analyser (FIAflow2, Burkard Scientific, Uxbridge, UK).

In June and December, soil samples were taken from the top 10 cm of soil using a 2-cm diameter soil corer. NO_3^- and NH_4^+ was then extracted by shaking 5 g dry weight equivalent of soil in 50 mL 2 M KCl for 30 min. Samples were filtered through Whatman No. 42 ashless filter paper and the NH_4^+ and NO_3^- content determined colorimetrically by flow injection analysis.

VEGETATION BIOMASS AND NITROGEN

Annual above-ground biomass production was determined from two harvests, first a clip to 2.5 cm above soil in June (across the whole community) and then a complete harvest to soil level on a single occasion in October within a 78-mm diameter circular sub-area. This measure therefore incorporates the production removed by the twice yearly 'management' cuts plus the biomass below the 2.5 cm cut level of those (i.e. the plant bases). Samples were oven dried (48 h at 60 °C) and weighed. N concentrations were determined from NH_4^+ content of Kjeldahl digested homogenized sub-samples (Allen 1989).

STATISTICAL ANALYSES

Effects of community composition were determined from one-way ANOVA (SPSS v12.0.1; SPSS, Chicago, IL). Within the ANOVA, contrasts were undertaken between each community and the 12-species community to determine whether any single community differed significantly from the most species rich community (4 forbs + 4 sedges + 4 grasses; question (ii)), while Tukey's tests were performed to determine differences between all communities (question (i)). Contrasts were considered necessary since when specifically testing for differences from the most species rich community, the large number of comparisons made in the conservative Tukey's test (when comparing all communities) runs the risk of wrongly rejecting the hypothesis that differences exist between any one community and the most species rich community.

Effects of functional group richness (*FGR*), and species per functional group (*Sp*/*FG*) were determined by Type I Sum of Squares General Linear Model (GLM) with community composition nested within the fixed factors of *FGR* and *Sp*/*FG*. In this way, *F* values for *FGR* and *Sp*/*FG* are calculated by dividing their mean squares (MS) by the community composition MS (i.e. 'community' becomes the error term for *FGR* and *Sp*/*FG*). This minimizes the potential for community composition to confound effects of *FGR* and *Sp*/*FG* (i.e. since different levels of biodiversity are represented by different communities; Schmid *et al*. 2002). Effects of community composition, *FGR* and *Sp*/*FG* on NO_3^- and NH_4^+ concentrations at all sampling points across the year were analysed using repeated measures GLM.

Soil NO_3^- and NH_4^+, biomass and tissue N stocks were $\log_{10}$ + 1-transformed while analysis of leached NO_3^- and NH_4^+ was undertaken on rank transformed data (Conover & Iman 1981; Quinn & Keough 2002).

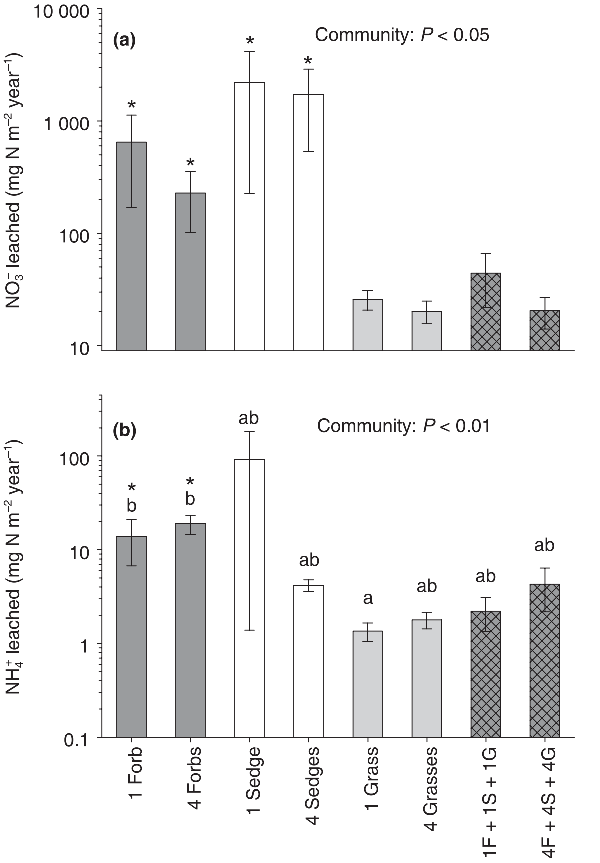

Fig. 1. Total leached losses of (a) NH_4^+ and NO_3^- over 1 year from eight different plant communities. *P* values indicate significance of community composition effects from ANOVA. Bars sharing the same letter are not statistically different ($P < 0.05$, Tukey's HSD comparing all communities). Where letters are not shown, no statistical differences occur between any pairs of communities. *indicates communities significantly different from the 12-species community (contrasts within ANOVA). No significant effects of *FGR*, or *Sp*/*FG* ($P > 0.05$). Error bars are 1 SE.

Results

LEACHING OF INORGANIC N

The greatest inter-community differences for all the parameters measured were seen in the total amounts of nitrate (NO_3^-) and ammonium (NH_4^+) leached (NO_3^- d.f. = 7,24, $F = 2.73$, $P < 0.05$; NH_4^+ d.f. = 7,24, $F = 3.73$, $P < 0.01$) (Fig. 1) with amounts leached from sedge and forb communities being a considerable one or two orders of magnitude greater than amounts leached from grass and mixed functional group

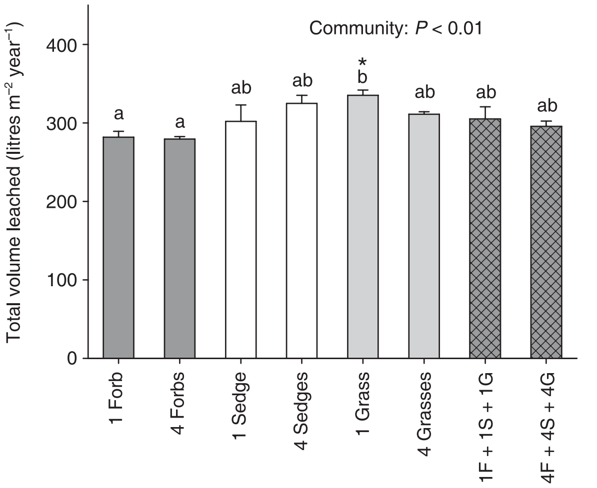

Fig. 2. Total volume of leachate lost from eight different plant communities. Statistical tests as for Fig. 1. No significant effects of *FGR* or *Sp*/*FG* ($P > 0.05$).

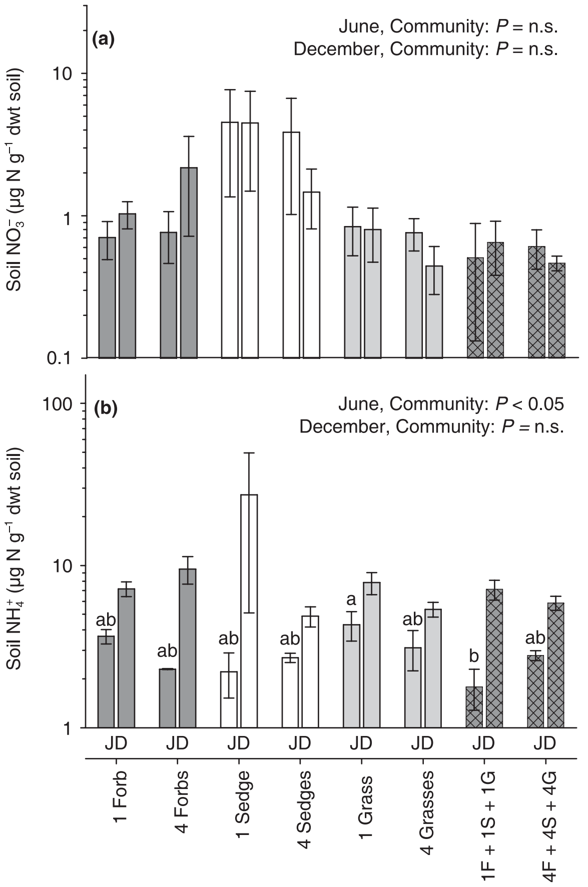

Fig. 3. Extractable nutrient concentrations of (a) NO_3^- and (b) NH_4^+ in soils from eight different plant communities in both June (J) and December (D). Statistical tests as for Fig. 1. Tukey comparisons in (b) for June only. No significant effects of *FGR* or *Sp*/*FG* ($P > 0.05$).

communities. NO_3^- leaching from sedge and forb communities (both 1- and 4-species) was significantly greater than the most species rich 4F + 4S + 4G community (contrast $P < 0.05$), while this was also true for NH_4^+ leaching from the forb communities (contrast $P < 0.05$). It was also apparent that the mixed functional group communities (1F + 1S + 1G and 4F + 4S + 4G) leached similar amounts of NO_3^- and NH_4^+ as the grass only communities despite containing forbs and sedges which showed high NO_3^- and NH_4^+ leaching when grown alone.

Our replicated communities also revealed surprisingly large variation in leaching (particularly of NO_3^-) within sedge-only and forb-only communities. For this reason, these communities were not significantly different in NO_3^- leaching when compared to other communities in Tukey's multiple comparison tests despite the order of magnitude differences between means (Fig. 1a).

Leachate volumes were much more similar between communities than for other parameters measured (Fig. 2). However, there were significant inter-community differences (d.f. = 7,24, $F = 3.14$, $P < 0.05$) with 1- and 4-forb communities leaching significantly lower volumes than the 1-grass communities (Tukey's $P < 0.05$). Leaching from the 1-grass community was significantly greater than from the most species rich 4F + 4S + 4G communities (contrast $P < 0.05$).

There were no overall effects of functional group richness or species per functional group on total amounts of NO_3^- and NH_4^+ leached or on total leachate volume.

Repeated measures analysis of NO_3^- and NH_4^+ concentrations in leachate throughout the year revealed a significant effect of community type (NO_3^- d.f. = 7,24, $F = 2.61$, $P < 0.05$; NH_4^+ d.f. = 7,24, $F = 7.91$, $P < 0.001$) (confirming the differences seen for total amounts leached) and a significant community × time interaction (NO_3^- d.f. = 49,168, $F = 2.30$, $P < 0.05$; NH_4^+ d.f. = 49,168, $F = 1.96$, $P < 0.01$) (see Fig. S1 in Supplementary material). As with annual amounts leached, the mixed functional group communities leached similarly low concentrations of NO_3^- as the grass communities. Generally, communities where each functional group was represented by one species showed similar seasonal patterns of NO_3^- and NH_4^+ concentrations as communities where each functional group was represented by four species. The exception was the sedge communities, where leached NO_3^- concentrations were considerably higher from July to January in the 4-sedge communities than in the 1-sedge communities (the 1-sedge communities showing greater NO_3^- concentrations from March to June). Leached NH_4^+ concentrations also showed a considerable peak from January to March in the 1-sedge communities that was not seen in the 4-sedge communities. There were no overall effects of functional group richness or species per functional group.

EXTRACTABLE SOIL NITROGEN

Concentrations of NO_3^- and NH_4^+ in soil for both June and December measurements also showed large differences between communities but these differences were only statistically significant for NH_4^+ in June (d.f. = 7,24, $F = 2.52$, $P < 0.05$) (Fig. 3). Again there was large variation within communities, and only 1-grass and 1F + 1S + 1F communities (June NH_4^+) were significantly different in multiple comparison tests. However, the pattern of differences between communities for

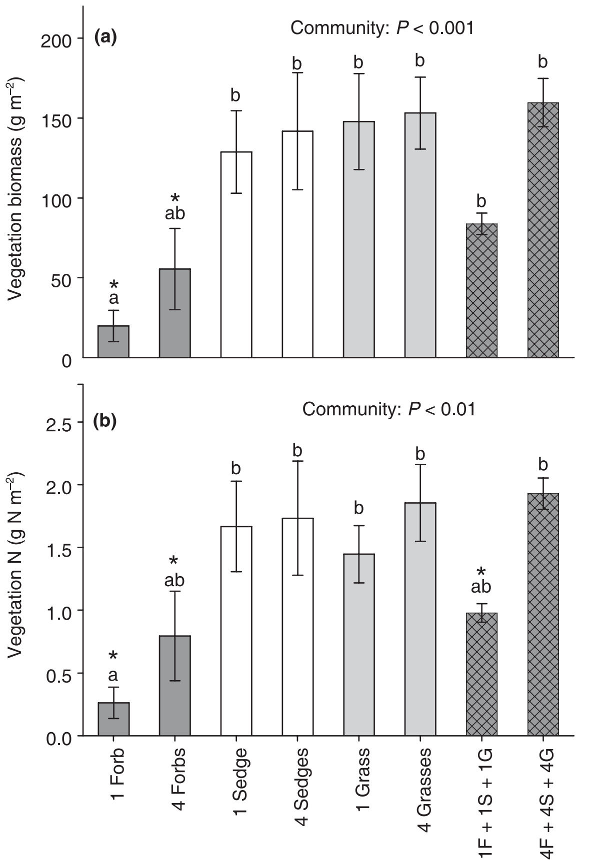

Fig. 4. Above-ground biomass (a), and total above-ground vegetation N (b) in eight different plant communities. Statistics are as for Fig. 1. No significant effects of *FGR* or *Sp/FG* ($P > 0.05$).

soil NO_3^- and NH_4^+ did somewhat reflect the pattern seen for leached NO_3^- and NH_4^+. There were no effects of functional group richness or species per functional group.

VEGETATION BIOMASS PRODUCTION AND NUTRIENT CONTENT

There were clear differences between communities in vegetation biomass (d.f. = 7,24, $F = 5.83$, $P < 0.001$) and N stocks (d.f. = 7,24, $F = 5.06$, $P < 0.01$) (Fig. 4). Here, forb communities tended to have the lowest productivity and smallest quantity of biomass N while these were greater in (and very similar between) the sedge, grass and the most species rich (4F + 4S + 4G) communities. Biomass was significantly lower compared to the most species rich community in 1- and 4-forb communities, while N stocks were significantly smaller in these plus the 1F + 1S + 1G community (compared to the most species rich community) (contrast $P < 0.05$). There were no overall effects of functional group richness or species per functional group on biomass production or the amount of N contained within that biomass.

Discussion

This study has revealed that the major plant components of ancient limestone grassland can differ by a considerable two-orders-of-magnitude in their capacity to retain inorganic N and prevent high concentrations in leachate. They also show smaller, but still significant and important differences in their provision of other ecosystem services (water retention, productivity and soil and plant nutrient status). In contrast to the differences between plant communities consisting of different plant functional groups, we found no evidence of a benefit to ecosystem services of mixing functional groups or of increasing the number of species representing each functional group.

EFFECTS OF COMMUNITY COMPOSITION ON N LEACHING

Comparison of the single functional group communities provides insight into how the dominant plant components may influence N leaching. In particular, the very low N leaching from grass communities show that these plant components of ancient limestone grassland have a considerable capacity to retain inorganic N and limit its concentration in leachate. Indeed, since grass-only communities leach as little N as mixed functional group communities, it is possible that grasses dominate the leaching characteristics of the ancient limestone grassland at Cressbrookdale such that mixed communities simply have low N leaching because they contain grasses. This may also be true for extractable soil N, which was equally low in both grass-only and mixed functional group communities. This suggests that grasses play the dominant role in the N dynamics of the system and are the most important component of the vegetation for maintaining N accumulation in the face of pressure from N deposition. These findings are also consistent with the work of Cardinale *et al.* (2006) who have shown that the functioning of the most species rich polyculture may be no different from that of the highest functioning single species.

Provision of these services by grasses may be partially explained by their high nutrient conservation and long leaf life, such that more nutrients are retained above-ground and less returned to soil (Aerts & Chapin 2000). Note also that lower tissue N concentrations of sedges and grasses in this study (20.7 and 22.6 mg g^{-1} on average) are consistent with their slower turnover and greater nutrient retention compared to the more nutrient rich forbs of this study (tissue N of 26.7 mg g^{-1}). The fine root systems of grasses may also contribute since root biomass has been seen to negatively correlate with NO_3^- leaching in previous studies (Scherer-Lorenzen *et al.* 2003). While we have not quantified root biomass, we would expect the fine, highly branched root systems characteristic of grasses to more efficiently exploit the soil compared to the simpler 'tap root' systems of forbs, as has been observed in other grassland (Levang-Brilz & Biondini 2003).

Although their more-simple root architecture may partially explain why forb-only communities show much higher levels of N leaching, other factors such as phenology may also play a role. The increases in leachate NO_3^- concentrations seen from November onwards in forb communities, for instance, are consistent with the complete foliar senescence on first frost of the forb *Leontodon hispidus* (the forb within the 1-forb communities). While the other forbs in the 4-forb communities do not show such complete die-back, there is still a tendency for forbs from limestone grassland to lose more leaf biomass in winter compared to other functional groups (Furness 1978; Grime 2002). Therefore, forbs other than *L. hispidus* may still contribute to the greater winter leaching of N from forb communities.

The particularly high rates of N leaching from sedge communities is harder to explain since these plants have high nutrient conservation and long-lived foliage (i.e. high retention of N above-ground) and so might be expected to establish a slow N turnover, low N status system similar to the grass communities. One mechanism explaining the greater loss of N from sedge communities compared to non-sedge communities is the development of mycorrhizal mycelial networks. Unlike the sedges, the grasses and forbs used in the mesocosms benefit from forming arbuscular mycorrhizas (Johnson *et al*. 2004), which enable plants to increase uptake of N and P (Hodge *et al*. 2001; Johnson *et al*. 2001). Greater uptake of P is likely to lead to higher N use efficiency because this limestone grassland is co-limited by N and P. Also, the sedge communities retained large inter-plant spaces between individual plants despite being established for more than 8 years. Soil-only mesocosms in our experiment (not reported here) which only contained the standard moss layer had by far the highest levels of leachate and soil NO_3^- (*c*. 10-fold more than the sedge communities, possibly as a result of high rates of nitrification). This may suggest that sedge communities were acting as partially planted, partially unplanted systems to produce large amounts of NO_3^-.

It is also apparent that in these communities – and the forb only communities – large variation exists between mesocosms constructed of the same species. These large differences cannot be the result of any sampling effect (Fridley 2001) since the communities are identical replicates. This highlights the extent to which variation in function can occur despite identical composition.

Despite the higher N leaching from sedge and forb communities, we do not suggest that these functional groups are of no value in maintaining low N leaching in limestone grassland. For instance, in a more heterogeneous and disturbed 'real' limestone pasture (compared to our mesocosms), there may be greater value in having a mix of functional groups with their diversity of rooting depth, rooting pattern and N demand.

IMPACTS OF COMMUNITY CHANGE ON SOIL NITROGEN AND FODDER PRODUCTION

While this study set out to focus on inorganic N leaching, it also revealed the importance of community composition and the contribution of major plant components to soil N and biomass production and nutrient status (fodder production and quality). Soil NO_3^- and NH_4^+ showed similar patterns and magnitudes of inter-community differences as leachate N, indicating that community impacts on soil N may be significant drivers of leachate N. In contrast, the pattern of inter-community differences for biomass production and biomass N were rather different to leachate N. The forb-only communities, in particular, showed smaller productivity and biomass N compared to most other communities; there was also a surprising similarity in biomass and vegetation N stocks between the grass, sedge and the most floristically rich 4F + 4S + 4G communities.

Hooper & Vitousek (1997, 1998) and Dukes (2001) have previously directly compared ecosystem functioning between communities consisting of different plant functional groups that have been replicated enough to allow direct statistical comparison between communities (i.e. in addition to the emergent relationships of species or functional type richness with function that have been reported in many studies). In these experiments, the magnitude of inter-community differences in above-ground biomass (reported by both) are comparable to our study, but differences in soil nutrients and leaching (reported in the Hooper and Vitousek studies) were considerably smaller (i.e. within the same order of magnitude) than those reported by us (more than two orders of magnitude). Further, the high level of NO_3^- leaching from our forb-only and particularly the sedge-only communities contrasts with findings of a past biodiversity–function leaching study using communities modelled on European grasslands (Scherer-Lorenzen *et al*. 2003). In that study, most communities (without legumes) utilized inorganic N so efficiently that almost no NO_3^- leaching occurred. It could be argued that the difference in duration of the past two studies compared to ours may contribute to those differences (the previous studies reported results within 2 years, us after 8 years) and that this highlights the importance of using well-established, mature systems in experimental communities. However, since these studies are based on different grassland systems, those differences could simply be down to taxonomic, climatic and edaphic differences. With more certainty, the maturity of our communities may explain the greater magnitude of community differences seen in NO_3^- leaching from our experiment reported here after 8 years compared to when our study was in its fourth year. In that earlier analysis, we found that communities differed by just one order of magnitude at most in NO_3^- leaching (see Fig. 111 in Grime 2001; Booth 2002).

IMPORTANCE OF BIODIVERSITY FOR PROVISION OF ECOSYSTEM SERVICES IN LIMESTONE GRASSLAND

We found no evidence that mixing functional groups or increasing the number of species representing each functional group promotes ecosystem function in limestone grassland. The absence of a functional group mixing effect contrasts with the importance of functional group richness often observed in past studies (e.g. Hooper & Vitousek 1997; Tilman *et al*. 1997; Spehn *et al*. 2005). However, the idiosyncratic nature of biodiversity relationships with various measures of ecosystem function is already well known, even among experiments with relatively similar design (Hector *et al*. 2007) and so contrasting findings from experiments on different communities are not unexpected. We also acknowledge that since a major part of our experimental design was to determine differences between major component plants and communities of known composition, our experimental design is not as strong at drawing out functional group richness effects compared to other studies where species and functional group richness are the main experimental variables. None-the-less, we note that even a re-analysis of our data using a standard two-way ANOVA (with *FGR* and *Sp*/*FG* as fixed factors) without fitting community composition finds only a single *FGR* effect: that outcome occurs despite this approach giving the greatest chance of revealing *FGR* and *Sp*/*FG* effects. In that case, mixing functional groups reduces NO_3^- leaching, an effect which is attributable to the dominant role played by grasses (as already noted above) such that the mixed functional group communities leach as little NO_3^- as the grass only communities.

ENVIRONMENTAL CHANGE AND VEGETATION CHANGE IN ANCIENT LIMESTONE GRASSLAND: CONSEQUENCES FOR ECOSYSTEM FUNCTION

While our study does not directly test the impacts of actual shifts in community structure, the clear importance of grasses in maintaining low N leaching rates indicates that the loss of forbs or sedges from grasslands which are likely to occur as a consequence of N deposition or agricultural improvement (Bobbink *et al.* 1998; Preston *et al.* 2002) will not reduce the capacity of ancient limestone grassland to retain inorganic N. We cannot, however, say whether that will hold true for organic N. Our work also suggests that fodder production and quality (biomass and biomass N) is also unlikely to suffer unless communities shift towards forb dominated systems (a change not expected under any likely environmental change scenario). Further, since all three functional groups in these grasslands are well represented (with an approximate abundance ratio for grasses : sedges : forbs of 2 : 1 : 2) even unexpected shifts – such as towards forb dominance – would have to be major to result in reduced provision of ecosystem services.

On a similar note, we highlight that the analyses of leachate NO_3^- concentrations throughout the year show that no community produces NO_3^- concentrations above the European standard for drinking water (50 mg N L^{-1}, or 1.7 on the $\log_{10}$ + 1-scale of Fig. S1) indicating that previously reported concerns for leaching above this threshold by some communities in a past biodiversity–function grassland study (Scherer-Lorenzen *et al.* 2003) do not apply here. We therefore suggest that while increased diversity has been proposed as a mechanism to reduce NO_3^- leaching from grasslands (Scherer-Lorenzen *et al.* 2003), so long as limestone grasslands continue to contain an abundance of grass, even very low levels of diversity can ensure a low concentration of NO_3^- in leachate.

In respect to this, Hector & Bagchi (2007) have recently provided analysis showing that biodiversity should be maintained for ecosystem multifunctionality – that is, since different species influence different functions, maintaining high species diversity should help promote multiple functions. This has also been proposed as the 'multivariate dominance effect' by Duffy *et al.* (2003). For limestone grassland, our work does indeed show that the most species rich community performs well in reducing inorganic N leaching, retaining water and maintaining productivity. Only soil N is 'low' in that community compared to the highest single species community, but this also may be considered desirable given that low soil fertility is needed to maintain community composition and low leaching rates. The most species rich community may therefore be seen as the safe 'insurance option' for maintaining most ecosystem services well. However, the community that generally does as well as (or a little better than) the most species rich community is always the 4-grass community, suggesting that grasses play an important role in promoting all the functions measured (not just leaching), rather than different functions being promoted by different functional groups. This again leads us to conclude that as long as limestone grasslands continue to contain an abundance of grass, even low levels of diversity can ensure maintenance of the ecosystem functions measured. Of course, the meta-analysis of Hector & Bagchi (2007) includes many systems and many functions, and we cannot suggest that every possible function within limestone grassland is maintained equally well by grasses as by the most species rich community.

Conclusion

This study has revealed up to two orders of magnitude differences in the capacities of the dominant plant functional groups to provide ecosystem services within ancient limestone grassland. In particular, grasses appear to play a dominant role in maintaining low inorganic N leaching rates, and are likely to be most important in providing the service of clean groundwater provision. Grass communities also appear to perform equally well as the most species rich communities for the other ecosystem services measured. Further, we find no evidence that any one ecosystem function benefits from increased functional group richness or species per functional group. Overall, we conclude that expected shifts in community composition expected under N pollution may not severely impact on N leaching, water retention, fodder production or soil nutrient status in what is one of the most floristically rich ecosystems in northern Europe.

Acknowledgements

This work was partially funded by the Natural Environmental Research Council (grant GST/02/2683). GKP and DJ are currently supported by an RCUK Academic Fellowship and a NERC Advanced Fellowship, respectively.

References

Aerts, R. & Chapin, III F.S. (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research*, **30**, 1–67.

Allen, S.E. (ed.) (1989) *Chemical Analysis of Ecological Materials*, 2nd edn. Blackwell Scientific, Oxford.

Bennie, J., Hill, M.O., Baxter, R. & Huntley, B. (2006) Influence of slope and aspect on long-term vegetation change in British chalk grasslands. *Journal of Ecology*, **94**, 355–368.

Bobbink, R., Hornung, M. & Roelofs, J.M. (1998) The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, **86**, 717–738.

Booth, R.E. (2002) *The role of diversity in the maintenance of community and ecosystem function; intra and inter specific diversity*. PhD thesis, University of Sheffield, UK.

Cardinale, B.J., Srivastava, D.S., Duffy, J.E., Wright, J.P., Downing, J.E., Sankaran, M. & Jouseau, C. (2006) Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature*, **443**, 989–992.

Conover, W.J. & Iman, R.L. (1981) Rank transformations as a bridge between parametric and nonparametric statistics. *American Statistician*, **35**, 124–129.

Da Vies, J., Briarty, L.G. & O'Rieley, J. (1973) Observations on the swollen lateral roots of the cyperaceae. *New Phytologist*, **72**, 167–173.

Duffy, J.E., Richardson, J.P. & Canuel, E.A. (2003) Grazer diversity effects on ecosystem functioning in seagrass beds. *Ecology Letters*, **6**, 637–645.

Dukes, J.S. (2001) Productivity and complementarity in grassland microcosms of varying diversity. *Oikos*, **94**, 468–480.

Fridley, J.D. (2001) The influence of species diversity on ecosystem productivity: how, where, and why? *Oikos*, **93**, 514–526.

Furness, S.B. (1978) *Ecological Investigations of Growth and Temperature Responses in Bryophytes*. PhD Thesis, University of Sheffield, UK.

Grime, J.P. (2001) *Plant Strategies, Vegetation Processes and Ecosystem Properties*, 2nd edn. Wiley, Chichester.

Grime, J.P. (2002) Declining plant diversity: empty niches or functional shifts? *Journal of Vegetation Science*, **13**, 457–460.

Hadi, D.A. & Cape, J.N. (1995) Preservation of throughfall samples by chloroform and thymol. *International Journal of Environmental Analytical Chemistry*, **61**, 103–116.

Harley, J.L. & Harley, E.L. (1987) A checklist of mycorrhiza in the British flora. *New Phytologist*, **105** (Suppl.), 1–102.

Hector, A. & Bagchi, R. (2007) Biodiversity and ecosystem multifunctionality. *Nature*, **448**, 188–190.

Hector, A., Joshi, J., Scherer-Lorenzen, M., Schmid, B., Spehn, E.M., Wacker, L. *et al*. (2007) Biodiversity and ecosystem functioning: reconciling the results of experimental and observational studies. *Functional Ecology*, **21**, 998–1002.

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G. *et al*. (1999) Plant diversity and productivity experiments in European grasslands. *Science*, **286**, 1123–1127.

Hodge, A., Campbell, C.D. & Fitter, A.H. (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature*, **413**, 297–299.

Hooper, D.U. & Vitousek, P.M. (1997) The effects of plant composition and diversity on ecosystem processes. *Science*, **277**, 1302–1305.

Hooper, D.U. & Vitousek, P.M. (1998) Effects of plant composition and diversity on nutrient cycling. *Ecological Monographs*, **68**, 121–149.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S. *et al*. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.

Johnson, D., Leake, J.R. & Read, D.J. (2001) Novel in-growth core system enables functional studies of grassland mycorrhizal mycelial networks. *New Phytologist*, **152**, 555–562.

Johnson, D., Vandenkoornhuyse, P.J., Leake, J.R., Gilbert, L., Booth, R.E., Grime, J.P., Young, J.P.W. & Read, D.J. (2004) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. *New Phytologist*, **161**, 503–515.

Lanta, V. & Leps, J. (2006) Effect of functional group richness and species richness in manipulated productivity-diversity studies: a glasshouse pot experiment. *Acta Oecologica*, **29**, 85–96.

Levang-Brilz, N. & Biondini, M.E. (2003) Growth rate, root development and nutrient uptake of 55 plant species from the Great Plains Grasslands, USA. *Plant Ecology*, **165**, 117–144.

Mulder, C.P.H., Jumpponen, A., Hogberg, P. & Huss-Danell, K. (2002) How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. *Oecologia*, **133**, 412–421.

Naeem, S., Thompson, L.J., Lawler, S.P., Lawton, J.H. & Woodfin, R.M. (1994) Declining biodiversity can alter the performance of ecosystems. *Nature*, **368**, 734–737.

Naeem, S., Thompson, L.J., Lawler, S.P., Lawton, J.H. & Woodfin, R.M. (1995) Empirical-evidence that declining species-diversity may alter the performance of terrestrial ecosystems. *Philosophical Transactions of the Royal Society of London, Series B*, **347**, 249–262.

Phoenix, G.K., Booth, R.E., Leake, J.R., Read, D.J., Grime, J.P. & Lee, J.A. (2003) Effects of enhanced nitrogen deposition and phosphorus limitation on nitrogen budgets of semi-natural grasslands. *Global Change Biology*, **9**, 1309–1321.

Phoenix, G.K., Leake, J.R., Read, D.J., Grime, J.P. & Lee, J.A. (2004) Accumulation of pollutant nitrogen in calcareous and acidic grasslands: evidence from N flux and ^{15}N tracer studies. *Water Air and Soil Pollution: Focus*, **4**, 159–167.

Preston, C.D., Telfer, M.G., Arnold, H.R., Carey, P.D., Cooper, J.M., Dines, T.D., Hill, M.O., Pearman, D.A., Roy, D.B. & Smart, S.M. (2002) *The Changing Flora of the UK*. DEFRA, London.

Quinn, G.P. & Keough, M.J. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

Scherer-Lorenzen, M., Palmborg, C., Prinz. A. & Schulze, E.D. (2003) The role of plant diversity and composition for nitrate leaching in grasslands. *Ecology*, **84**, 1539–1552.

Schmid, B., Hector, A., Huston, M.A., Inchausti, P., Nijs, I., Leadley, P.W. & Tilman, D. (2002) The design and analysis of biodiversity experiments. Biodiversity and Ecosystem Functioning. *Biodiversity and Ecosystem Functioning: Synthesis and Perspectives* (eds M. Loreau, S. Naeem & P. Inchausti), Oxford University Press, Oxford, pp. 61–75.

Spehn, E.M., Hector, A., Joshi, J., Scherer-Lorenzen, M., Schmid, B., Bazeley-White, E. *et al*. (2005) Ecosystem effects of biodiversity manipulations in European grasslands. *Ecological Monographs*, **75**, 37–63.

Symstad, A.J. (2000) A test of the effects of functional group richness and composition on grassland invasibility. *Ecology*, **81**, 99–109.

Thompson, K., Askew, A.P., Grime, J.P., Dunnett, N.P. & Willis, A.J. (2005) Biodiversity, ecosystem function and plant traits in mature and immature plant communities. *Functional Ecology*, **19**, 355–358.

Tilman, D., Naeem, S., Knops, J., Reich, P., Siemann, E., Wedin, D., Ritchie, M. & Lawton, J. (1997) The influence of functional diversity and composition on ecosystem processes. *Science*, **278**, 1866–1867.

Tilman, D., Reich, P.B. & Knops, J.M.H. (2006) Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature*, **441**, 629–632.

Tilman, D., Wedin, D. & Knops, J. (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*, **379**, 718–720.

Received 19 November 2007; accepted 24 April 2008
Handling Editor: Robert Jones

Supplementary material

The following supplemental material is available for this article:

Figure S1 Seasonal dynamics of NO_3^- and NH_4^+ concentrations in leachate.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2745.2008.01403.x
(This link will take you to the article abstract).

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.