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Ecologically sustainable fertility management for the maintenance of species-rich hay meadows: a 12-year fertilizer and lime experiment

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Summary

1. Increased use of artificial fertilizers has caused widespread loss of species-rich grasslands throughout Britain and mainland Europe. Species-rich meadows are traditionally managed by hay cutting, use of farmyard manure (FYM) and occasional liming, but sustainable fertility management to maintain their botanical diversity is ill defined.

2. This study measured vegetation responses to fertilizers and lime applied over 12 years to species-rich upland and lowland mesotrophic hay meadows in the UK. Treatments consisted of three rates of FYM applied annually or triennially, inorganic fertilizers giving equivalent amounts of N, P and K to two of the annual and two of the triennial FYM treatments, and lime applied either alone or with annual or triennial FYM.

3. Farmyard manure at 24 tonnes ha^{-1} year^{-1} reduced total species richness and the richness of positive indicator species at both meadows and increased aggregate cover of negative indicator species. Lower rates of FYM application were also detrimental at the lowland meadow, but not at the upland one. Inorganic fertilizers were no more damaging to plant species richness than equivalent FYM treatments.

4. At the upland meadow, vegetation quality was maintained by continuing past FYM inputs (12 t ha^{-1} year^{-1}), but improved at lower rates. At the lowland meadow, which has no recent history of fertilizer use, rates equivalent to only ≤4 tonnes FYM ha^{-1} year^{-1} were sustainable. Evidence was slight of vegetation adapting to increased inputs at either meadow. Between-meadow differences in vulnerability to treatments apparently reflected differences in site-specific factors, particularly past management, rather than differences in plant community type.

5. *Synthesis and applications*. Relatively modest fertility inputs can reduce the ecological value of meadows with no recent history of such inputs, whereas moderate inputs of fertilizer and lime will be ecologically sustainable in meadows adapted to a long history of application. Decisions on sustainable levels of fertilizer use to maintain or enhance botanical diversity of grassland should be based on knowledge of soil physical and chemical status and past fertility management. Inorganic fertilizers are no more damaging than farmyard manure when applied at equivalent amounts of N, P and K.

Key-words: botanical diversity, farmyard manure, inorganic fertilizer, soil nitrogen, soil pH, soil phosphorus

Introduction

Agricultural intensification, particularly the increased use of artificial fertilizers, caused widespread losses of species-rich grasslands throughout Britain and mainland Europe during the second half of the 20th century (Fuller 1987; Ellenberg 1988). Fertilization reduces species richness in grasslands by enhancing competitive exclusion (Grime 1973), mainly through increased intensity of competition for light (Hautier, Niklaus & Hector 2009) and

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reduced seedling colonization (Tilman 1993), both resulting from increased above-ground biomass production (Socher *et al.* 2012). Many studies have shown that inorganic fertilizers reduce plant species richness in meadows (e.g. Mountford, Lakhani & Kirkham 1993; Kirkham, Mountford & Wilkins 1996; Silvertown *et al.* 2006).

Many species-rich meadows are traditionally managed by hay cutting, use of farmyard manure (FYM) and occasional liming (Ellenberg 1988; Rodwell 1992; Tallowin 1998; Crofts & Jefferson 1999; Jefferson 2005), yet sustainable practices to maintain their botanical diversity are still ill defined. Furthermore, no previous study has examined the impacts of FYM on vegetation when matched with inorganic fertilizers supplying equivalent amounts of nitrogen (N), phosphorus (P) and potassium (K). Changes in farming practice have reduced the availability of FYM, so there is a need to ascertain whether, and when, inorganic fertilizers can be substituted for FYM at equivalent levels of N, P and K content without reducing the species richness and botanical integrity of the vegetation.

Much of the focus on the effects of fertilizers in grassland has been on the amount of N applied. However, studies in north-western Europe have shown that P enrichment is generally a more important driver of reductions in species diversity than N (Ceulemans *et al.* 2011) and that high species richness occurs within a narrow band of low extractable soil P, in contrast to soil N and K (Janssens *et al.* 1998; Critchley *et al.* 2002a). Although it was not a specific objective of this study to investigate the relative importance of individual nutrients, these aspects are recognized in the analysis and interpretation of the results.

An experiment involving several FYM or matched inorganic equivalent fertilizer treatments and treatments incorporating initial liming was established in 1999 in species-rich meadows of two contrasting Arrhenatheratalia plant communities (Braun-Blanquet 1964) in the UK. The overall aim of the study was to identify ecologically sustainable fertilizer management for the maintenance of species diversity in species-rich meadows. Specifically, we set out to identify the following for the maintenance or enhancement of species richness and botanical composition: (i) optimum amounts and frequencies of FYM; (ii) any difference between FYM and equivalent amounts of N, P and K applied as inorganic fertilizers; (iii) the effect of liming to raise and/or maintain soil pH and any interaction with FYM use.

Earlier results (to 2005) from these sites were reported elsewhere (Kirkham *et al.* 2002, 2008), and this paper describes botanical responses over the period 1999–2010.

Materials and methods

EXPERIMENT SITES

Experimental plots were established in 1999 on an upland fringe species-rich meadow at 54° 27′ N and 2° 34′ W (altitude 250 m a.s.l.) near the village of Orton in Cumbria, north-west England, and at a lowland species-rich meadow close to Monmouth at 51° 46′ N and 2° 41′ W (altitude 190 m a.s.l.) in south Wales. Average rainfall (1999–2009) for upland and lowland sites, respectively, was 982 and 797 mm for annual rainfall and 525 and 462 mm, respectively, for April–October. These values were 3% and 6% higher than the longer term (1980–2009) averages for annual and seasonal rainfall, respectively, at the upland site, and 8% and 13% higher, respectively, at the lowland site.

Plant communities

The vegetation of the upland meadow in 1999 corresponded to the Geranio-sylvatici-Trisetetum Knapp 1951 association within the Triseto-polygonium alliance under the Zurich-Montpellier classification system (Braun-Blanquet 1964), and the lowland site to the Centaureo-Cynosuretum Br. Bl. & Tx. 1952 association within the Cynosurion-cristati alliance. Communities were defined more specifically (Kirkham *et al.* 2002) within the UK National Vegetation Community (NVC) system (Rodwell 1992): the upland site as MG3b (*Briza media*) sub-community of *Anthoxanthum odoratum-Geranium sylvaticum* (MG3) grassland; the lowland site as the MG5c (*Danthonia decumbens*) sub-community of MG5 (*Cynosurus cristatus-Centaurea nigra*) grassland, although lacking some of the preferential species differentiating MG5c from other sub-communities (Rodwell 1992).

Past management

Both meadows had a long history of cutting for hay, after 1 July (upland meadow) and after the second week in July (lowland meadow), with aftermath grazing by sheep at both sites. The upland meadow received about 12 t FYM ha^{-1} each year, usually in late April, and periodic applications of lime (amounts unknown), the last in about 1993. The lowland meadow had received no form of fertilizer or lime for at least 10 years, possibly much longer.

Soils

Soil texture was clay loam at the upland meadow and a mixture of sandy silt loam to silty clay loam at the lowland meadow. The upland meadow soils were notably higher in total N, carbon and organic matter, and slightly higher in extractable soil P, than the lowland meadow (see Table S1 in Supporting Information), with total soil N in particular at the lowland site lower than average for corresponding plant communities (Critchley *et al.* 2002b). Initial soil pH levels at both sites were notably lower than average for the relevant community types (Critchley *et al.* 2002b), whilst soil K levels were relatively high.

EXPERIMENTAL DESIGN

Farmyard manure and inorganic fertilizer treatments (Table 1) were applied between March and late April to individual 7 × 5 m plots in a randomized block design with three replicate blocks at each site. FYM treatments were chosen to encompass rates and frequencies traditionally applied to species-rich hay meadows in the UK (Crofts & Jefferson 1999). Access restrictions due to the national outbreak of foot and mouth disease (FMD)

Table 1. Treatments applied 1999–2010 at the upland and lowland meadows. Values are the mean amounts applied (kg ha^{-1} year^{-1} elemental N, P and K) either as farmyard manure (FYM, estimated) or in inorganic form (actual), averaged over 12 years (see text) 1999–2010. Treatments 13–15 were limed in both 1999 and 2005 at the upland site but in 1999 only at the lowland site. Treatments 2–12 received lime in 2005 only. Treatment 1 (established in 1999 at the lowland meadow and in 2005 at the upland meadow) received no lime or fertilizer in any year

	Upland meadow			Lowland meadow		
Treatment	N	P	K	N	P	K
1. Untreated control	0·0	0·0	0·0	0·0	0·0	0·0
2.*Limed (in 2005) control	0·0	0·0	0·0	0·0	0·0	0·0
3. FYM at 6 t ha^{-1} annual	4·4	5·0	34·5	2·9	3·8	29·2
4. § *FYM at 12 t ha^{-1} annual	8·8	10·0	68·9	5·7	7·6	58·5
5. §FYM at 24 t ha^{-1} annual	17·6	19·9	137·8	11·4	15·1	116·9
6. FYM at 6 t ha^{-1} triennially	1·5	2·2	11·3	0·9	1·3	11·0
7. §*FYM at 12 t ha^{-1} triennially	3·0	4·5	22·6	1·9	2·6	22·1
8. §FYM at 24 t ha^{-1} triennially	6·1	9·0	45·2	3·8	5·2	44·1
9. §Inorg. equivalent to Tr. 4	8·5	8·4	68·9	6·1	6·3	58·5
10. §Inorg. equivalent. to Tr. 5	17·0	16·8	137·8	12·1	12·5	116·9
11. §Inorg. equivalent to Tr. 7	3·0	3·6	22·6	2·4	2·1	22·1
12. §Inorg. equivalent to Tr. 8	6·1	7·2	45·2	4·8	4·2	44·1
13. *Lime in years 1 (and 7)	0·0	0·0	0·0	0·0	0·0	0·0
14. *Lime as Tr. 13 + FYM as Tr 4	8·8	10·0	68·9	5·7	7·6	58·5
15. *Lime as tr. 13 + FYM as Tr 7	3·0	4·5	22·6	1·9	2·6	22·1

Treatments comprising factorial series for fertilizer form × rate × frequency (FRF) or lime in 1999 × FYM frequency (LFF) are indicated by '§' and '*', respectively.

prevented treatment application in 2001, so that annual treatments received only 11/12 of the intended amounts over the 12-year period 1999–2010. Triennial treatments were applied in 1999, 2002, 2005 and 2008.

The methods and approaches used in relation to FYM analyses and liming are described in detail in Appendix S1. In summary, the FYM was sourced locally from the same farms each year and sampled at the time of application for analysis of organic and mineral N, total P and total K. Estimates of plant-available N supplied by FYM, both directly and subsequently mineralized within the soil, were improved from 2007 onwards, when the assumed availability of P was also increased from 60% to 80% of total P. The 1999–2010 mean estimates for the amount of N and P supplied by FYM (Table 1) were based upon revised estimates for the whole period whilst those for 'equivalent' inorganic fertilizer treatments were those actually applied, based upon annual estimates of the FYM NPK availability. Availability of K was assumed to be 90% throughout (Anon 2010).

Lime was applied to designated treatments (Table 1) at both sites in March–April 1999 and again in 2005 at the upland site and to all of the treatments in 2005 including previously untreated controls. Controls untreated throughout were also established at both sites, in 1999 at the lowland site and in 2005 at the upland meadow.

Plots were cut for hay after 1 July and after 15 July each year at the upland and lowland sites, respectively. The regrowth following the hay harvest was grazed along with the remainder of the field, using mature sheep from September–October until March–late April at the upland site and with cattle from mid-October/early November until late February at the lowland meadow.

Botanical assessments were carried out during May each year, except in 2001 at the upland meadow due to FMD restrictions. On each occasion, a single visual ground cover score was given for each species present within each of three randomly positioned 1-m^2 fixed quadrats per plot using three assessors, each assessing one of the three replicate blocks. As far as possible, the same assessors were used each year. Consistency of scoring between assessors was checked routinely each year by periodically comparing scores made independently on the same quadrat.

Soils were sampled in March 1999, 2002, 2004, 2007 and 2010 and analysed for pH, total N, total carbon, organic matter (loss-on-ignition), and for extractable P, K, magnesium (Mg) and calcium (Ca) (Appendix S1 gives more detail).

BOTANICAL VARIABLES

Four key variables were calculated using plot mean values for data analysis: total species richness (the number of vascular species per m^2); the number per m^2 of positive indicator (PI) species; the aggregate cover of these species as a percentage of total vegetation cover (PI%); and the aggregate cover of negative indicator (NI) species as a percentage of total vegetation cover (NI%). Both PI and NI species (Table S4) comprise those identified by Robertson & Jefferson (2000) for condition monitoring in UK statutory grassland sites in the relevant NVC communities (Rodwell 1992), with the NI list augmented by species associated with the negative effects of nutrient addition on plant community quality (Mountford, Lakhani & Kirkham 1993; Kirkham, Mountford & Wilkins 1996).

In addition, an Ellenberg fertility score was calculated for each quadrat as the mean fertility indicator value of the species present, weighted according to the proportional cover of each species. Species indicator values are on a nine point scale based upon the level of nutrient richness of the habitat with which a species is associated, derived from extensive survey data from mainland Europe (Ellenberg 1988) and modified for British conditions by Hill *et al.* (1999). Weighted fertility scores can therefore be a useful indicator of the level of past fertility management in grasslands (Smith *et al.* 2003; Kirkham *et al.* 2008). The mean score for vegetation at the upland meadow in 1999 was 4·47, within the ideal range of 4·33–4·67 identified by Smith *et al.* (2003) for this plant community. The mean score at the lowland meadow in 1999 was considerably lower at 4·0.

NI% and fertility scores were highly correlated at both sites (Pearson $r = 0{\cdot}845$ and $r = 0{\cdot}927$ at the upland and lowland sites, respectively, in 2010, both $P < 0{\cdot}001$). NI% was preferred for detailed analyses because it was overall more sensitive to treatments (weighted Ellenberg scores for 2010 are given in Table S13).

DATA ANALYSIS

All analyses were carried out using Genstat (Genstat 5 Committee 1997).

Treatment and treatment × time effects

At both sites, data for all years for each variable were analysed separately within each site, using repeated measures analysis of variance (ANOVA) at the lowland site, and at the upland site, in view of the missing year (2001), using repeated measures residual estimated maximum likelihood (REML). Three separate models were used in each case, with replicate blocks declared as a blocking factor in ANOVAS, and block + plot/year as a random effect in REML to give the equivalent structure. The first model included all treatments except untreated control plots established in 2005 at the upland meadow, the other two used balanced factorial subsets of treatments (Table 1): the form (FYM vs. inorganic) x rate x frequency (annual vs. triennial) (FRF) series; and the lime (1999 vs. 2005 only) × FYM frequency (LFF) series. All interactions, including with time, were tested within each series. The repeated measures aspect was accounted for within ANOVAS by adjusting all degrees of freedom in the year x treatment stratum for divergence from compound symmetry using the Greenhouse–Geisser epsilon correction factor, and within REML by using an antedependence (order 1) covariance model applied to the plot year residual term.

Percentage data were transformed before analysis by arcsine ($\sqrt{p}$), where p is the percentage expressed as a proportion, to ensure normality of residuals and homogeneity of residual variation. Further $\log_{10}$ transformation was required for some REML analyses.

Effects referred to in the description of results as 'significant' were significant at least at the $P < 0{\cdot}05$ level.

Mixed modelling

Data for 2010 for each site were analysed by mixed model analysis using REML to identify any effect of form, rate or frequency of fertilizer application additional to that simply accounted for by the mean amount of fertilizer applied per year, averaged over the whole study period. To account for the lack of equivalence of N and P supply between FYM treatments and corresponding inorganic treatments, and also for the absolute correlation across treatments at each site in the amounts of each element applied, two separate models were developed for each dependent variable tested (total species richness and PI species richness), using either applied N or P ha^{-1} year^{-1} as the fertilizer variable. In each case, the presence of any nonlinearity in response was tested by including a quadratic term (N^2 or P^2), or where more appropriate by $\log_n$-transformation of the explanatory variable. Nil fertilizer was used as one of the levels in each of the three fixed factors and block was included as a random factor.

Initial models included all main effects and interactions, with each term evaluated by a χ^2 test on the Wald statistic. The number and order of terms in the model were varied, but with N or P amount always included first, until a model was developed in which each term retained was significant ($P < 0{\cdot}05$). The model was then refined using the more conservative deviance test. Where the difference between the two forms of fertilizer lay primarily in a difference in the nonlinearity of response a refined model was tested with the P^2 (or N^2) x form term replaced by form nested within the quadratic term (e.g. P^2/form). This returned the two terms P^2 and P^2 × form, (Galwey 2006).

These analyses included data for all treatments limed in 2005 but not in 1999 (i.e. Trs 2–12).

Results

The overall treatment effect (averaged over all years) was significant in the all-treatment analyses for all variables at both sites except for PI% at the lowland site ($P = 0{\cdot}042$ for PI species richness at the upland site, $P < 0{\cdot}01$ or $P < 0{\cdot}001$ for the remainder – see Tables S5–12 for details of REML and ANOVA statistics). Year was highly significant in all analyses at both sites ($P = 0{\cdot}004$ within the LFF series at the lowland site, $P < 0{\cdot}001$ the remainder). Within the all-treatment analyses, the treatment x time interaction was significant only for PI% and NI% at the upland site but for all variables except PI% at the lowland site (which showed no effect of any factor other than time in any analysis). In other analyses, year x frequency of application was the most commonly significant interaction overall. Only NI% at the lowland site showed any significant effect of liming regime within the LFF series.

BOTANICAL CHANGE AND OVERALL TREATMENT EFFECTS 1999–2010

The upland meadow

Total species richness declined markedly under high rate annual FYM treatment (Tr. 5) (Fig. 1a). This treatment was significantly less species rich than all others when averaged over all years (note that probability levels cannot be assigned to within-treatment or within-year comparisons for this variable, since there was no significant treatment x time interaction). No other treatment showed any marked temporal trend in total species richness at this site, although within the FRF series, annual treatments were less species rich overall than triennial ones, and FYM treatments than their inorganic counterparts, both in total species richness (differences $P < 0{\cdot}001$ and $P = 0{\cdot}054$, respectively) and in richness of PI species ($P = 0{\cdot}025$ and $P = 0{\cdot}019$, respectively).

Positive indicator species richness, and particularly PI %, increased generally with time, but notably more slowly with annual FYM inputs at 12 and 24 t ha^{-1} (Trs. 4 and 5) (Fig. 1b,c). The difference in PI% compared to the unfertilized control was highly significant ($P < 0{\cdot}001$) by 2004 with Tr. 5 and by 2008 with Tr. 4, whilst with both treatments, differences in NI% compared to the control remained significant at this level from 2002 onwards. FYM enhanced NI% notably more than inorganic fertilizers ($P = 0{\cdot}002$ overall, FRF series), with the overall effects of both rate and frequency of application also marked within this analysis (medium < high and triennial < annual, both $P < 0{\cdot}001$ averaged over all years).

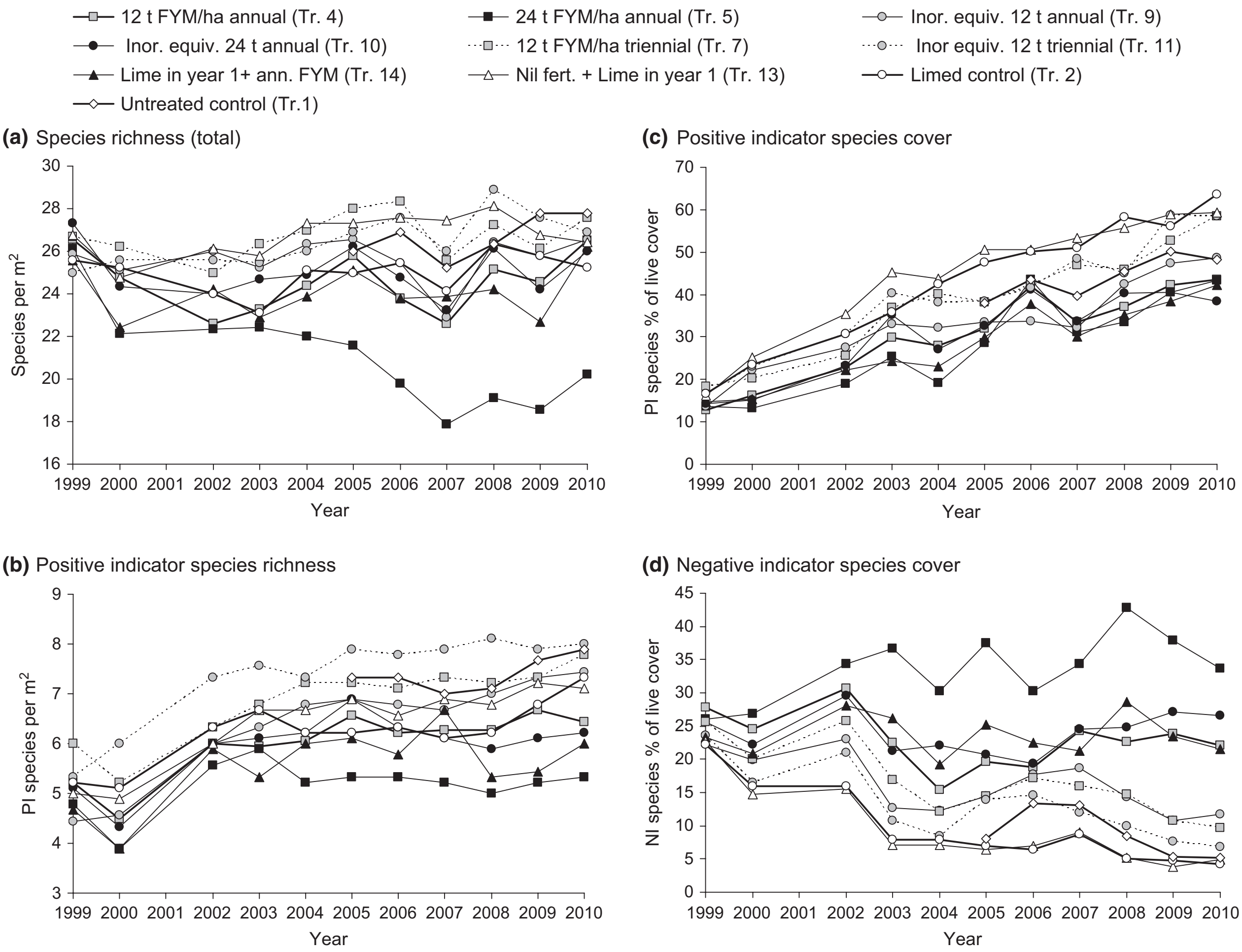

Fig. 1. Changes in vegetation composition at an upland meadow as influenced by some fertilizer and lime treatments. Note: all treatments received lime in 2005 except for the untreated control. FYM = farmyard manure.

The lowland meadow

Species richness declined progressively until 2009 with high rate annual FYM (Tr. 5): by 2006, this treatment was significantly less species rich than all others except Tr. 14 (annual FYM at 12 t ha^{-1} limed in 1999), diverging significantly from Tr. 14 by 2008 (Fig. 2a). The latter was significantly less species rich than unfertilized treatments (Trs. 1 and 2) in several years and averaged over all years, whilst the equivalent treatment not limed until 2005 (Tr. 4) declined after 2006 to a level lower than Tr. 14, though not significantly so (i.e. $P > 0{\cdot}05$). Liming alone, either in 1999 or 2005, had no discernible effect on species richness, and despite the trends shown by Trs. 4 and 14, the LFF series analysis showed no effect of liming regime, nor any interaction either with year or FYM application.

Both total species richness and PI species richness declined significantly after 2007 on untreated controls (Tr. 1), probably due to a series of late harvests during this period (Kirkham & Tallowin 1995). For both variables, however, the differences between Tr. 1 and Tr. 5 remained highly significant ($P < 0{\cdot}001$, except for PI richness in 2010: $P < 0{\cdot}05$).

Overall, FYM treatments reduced both total species richness and PI species richness more than their inorganic counterparts ($P = 0{\cdot}003$ and $P = 0{\cdot}002$, respectively, within the FRF series). PI species richness declined generally after 2005 (Fig. 2b), particularly with high rate annual FYM (Tr. 5), but only after 2006 with the lower rate (Tr. 4). Both these treatments were less PI rich than the corresponding inorganic treatment in most years. By contrast, PI% showed an overall decline between 1999 and 2005 across most treatments, and a general increase thereafter (Fig. 2c), but with no significant effect other than of time within any of the analyses.

NI% was initially much lower at the lowland than at the upland site, with an even more marked separation between treatments over time (Figs 1d and 2d), increasing markedly under annual fertilizer application (particularly Tr. 5) but remaining relatively unchanged under triennial and nil fertilizer treatments (Fig. 2d). NI% increased less markedly under inorganic treatments, and Tr. 5 differed from its inorganic counterpart (Tr. 10) in most years from 2004 onwards ($P < 0{\cdot}001$ in 2010). NI% increased more or less progressively with annual FYM at 12 t ha^{-1} until

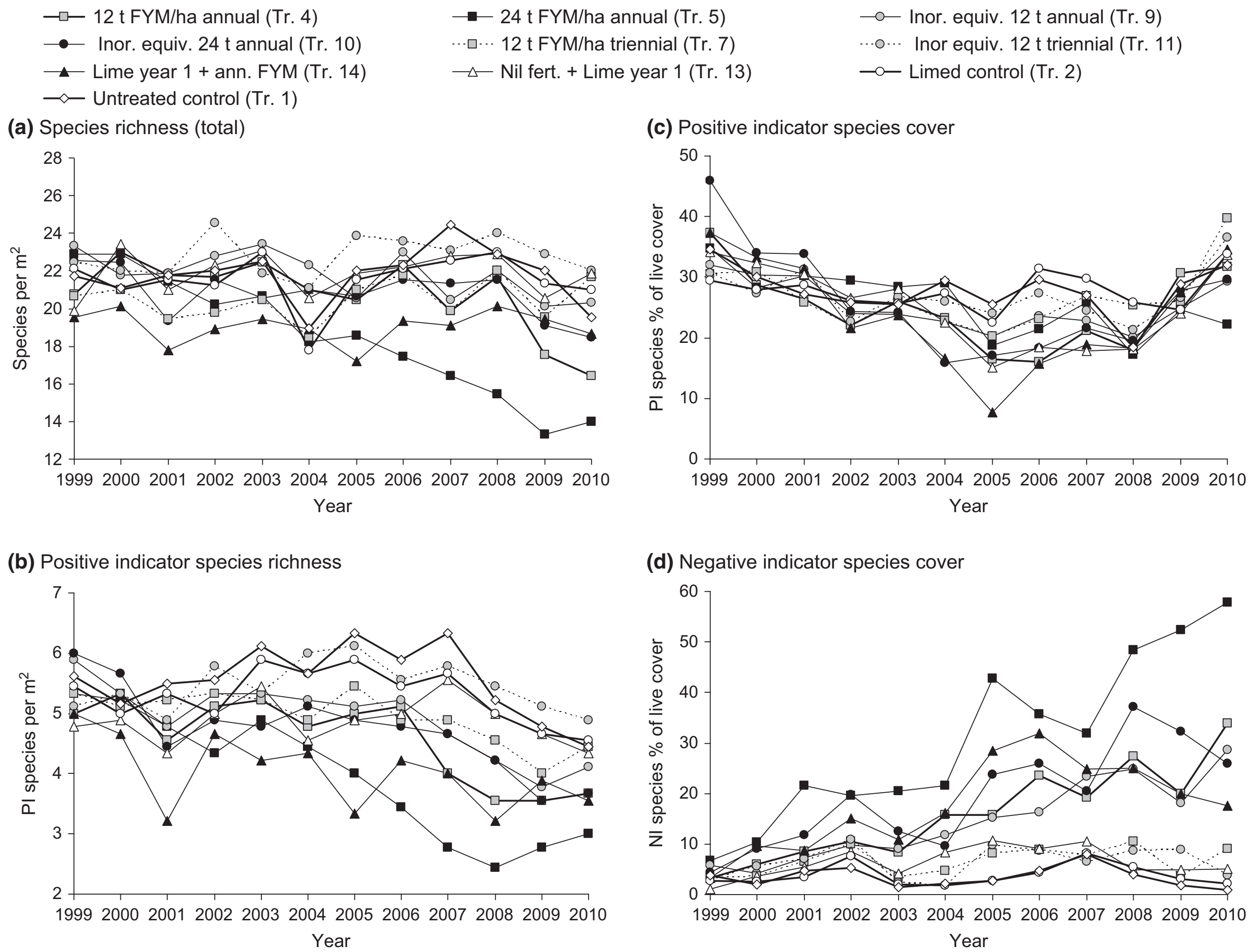

Fig. 2. Changes in vegetation composition at a lowland meadow as influenced by some fertilizer and lime treatments. Note: all treatments received lime in 2005 except for the untreated control treatment and those that had received lime in year 1. Treatments 1 and 2 were identical (nil input) until 2005. FYM = farmyard manure.

2006 (Trs. 4 and 14), particularly where lime had been applied in 1999 (Tr. 14). This trend continued under Tr. 4 but was reversed after 2006 under Tr. 14, so that the earlier significant difference between these two treatments was also reversed by 2010. These trends were reflected in an overall effect of form of fertilizer (FYM > inorganic, $P = 0{\cdot}008$) within the FRF factorial series and of liming regime within the LFF series ($P = 0{\cdot}042$).

MODELLED RELATIONSHIPS WITH AMOUNT OF FERTILIZER APPLIED

All the effects of rate and frequency of fertilizer application on both total species richness and PI species richness in 2010 at both sites were fully accounted for by the mean amount of N or P applied ha^{-1} year^{-1}, but a significant effect of form of fertilizer remained in most models. N and P models usually showed similar main effects, due to the complete correlation across treatments between the amounts of N and P applied. The form term was usually more significant in N than P models, reflecting a greater response per kg applied due to the much smaller discrepancies between FYM and inorganic 'equivalent' treatments in the amount of N applied compared to P (see Table 1). However, as P availability was considered to be the main limiting element at both sites, the main focus here is on P models (Fig. 3, see Fig. S1, for N models).

The final model for species richness in the upland meadow showed both a marked nonlinear response to fertilizer application (particularly FYM) and a marked difference in response between forms of fertilizer (Fig. 3a), the latter expressed mainly by a difference in the nonlinearity of response: a model with form nested within the squared term (P^2) was highly significant ($P < 0{\cdot}001$), with each term significant in the model ($P = 0{\cdot}010$, $P < 0{\cdot}001$ and $P = 0{\cdot}024$ for P, P^2 and $P^2 \times$ form, respectively, $P < 0{\cdot}001$ for the constant).

The overall relationship between PI species richness and nutrient input was linear at the upland meadow (Fig. 3b). Adding the form term improved the P model marginally ($P = 0{\cdot}081$), although this term was significant in the N model ($P = 0{\cdot}039$). The influence of nutrient amount (N or P) was highly significant in both models (both $P < 0{\cdot}001$), but with no significant interaction with form

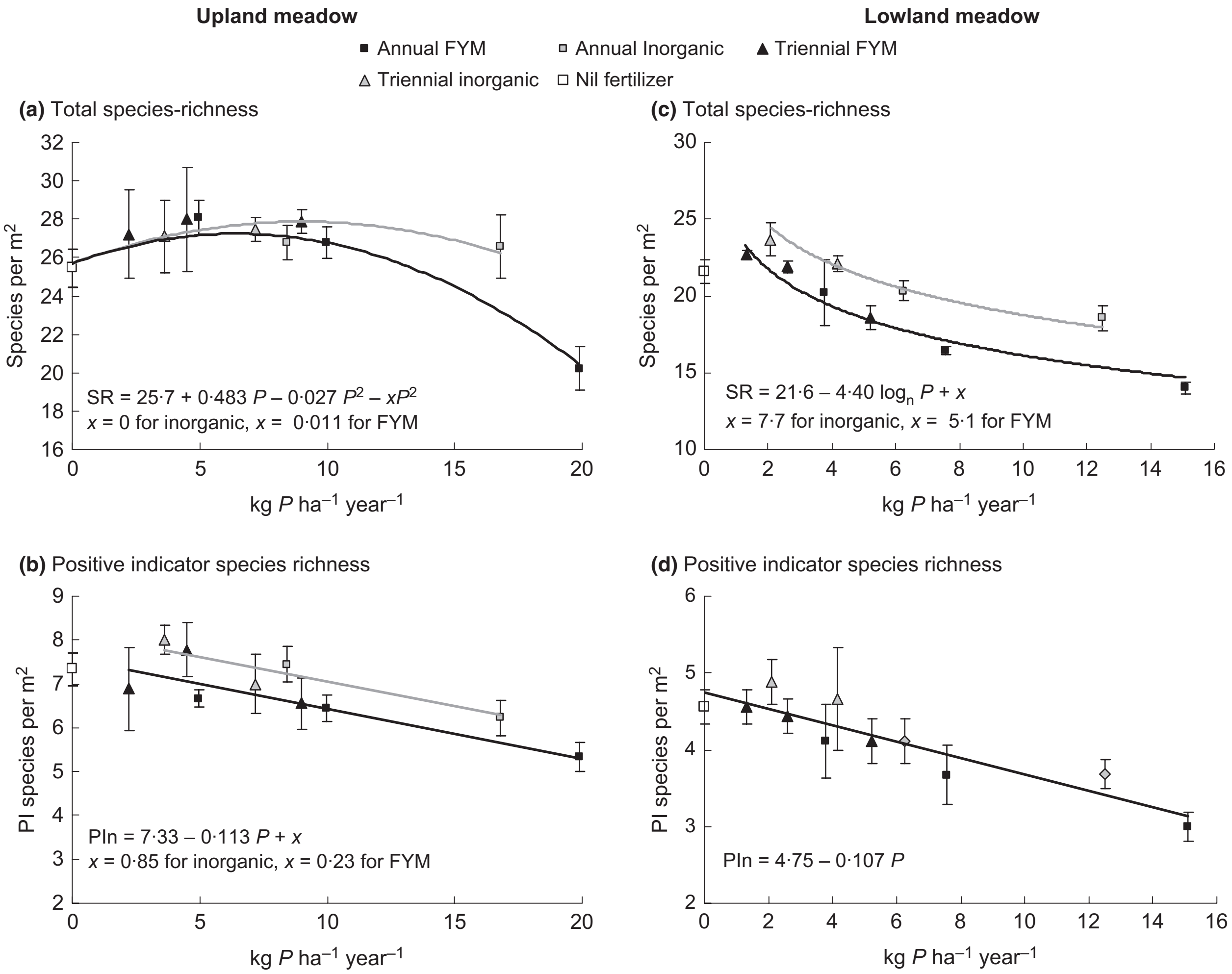

Fig. 3. Relationships between the amount of P applied per year (mean of 1999–2010) as either inorganic fertilizer or as farmyard manure (FYM) and: species richness (a and c); and the number of positive indicator species per m^2 (b and d). The lines or curves on each graph were fitted by REML with P as a continuous explanatory variable and with form of fertilizer as a factor. Error bars are ± standard errors of treatment means.

of fertilizer. Inorganic treatments were slightly more PI species rich at a given level of N or P input than FYM (by 0·75 and 0·62 PI species per m^2 in the N and P models, respectively). In both models, the predicted intercept values for inorganic fertilizers were higher than the actual limed control mean, reflecting a lack of response to inorganic fertilizers in the range equivalent to 0–8 kg P ha^{-1} $year^{-1}$.

Both FYM and inorganic fertilizer reduced species richness at the lowland meadow in a nonlinear fashion (Fig. 3c) best described by a linear relationship against $\log_n$P (or $\log_n$N) over the range of fertilizer treatments (but not nil fertilizer). The effect of form of fertilizer was very strong in each model ($P < 0{\cdot}001$), but there was no significant interaction, both models predicting a constant difference between inorganic and FYM treatments (+2·6 and +3·9 species m^{-2} for inorganic fertilizer in P and N models, respectively). However, the logarithmic relationship at the lowland site did not adequately describe the response at the lowest input levels compared to nil fertilizer, suggesting a humped pattern with the lowest fertilizer values at its peak (see Fig. 3c). A simple second- or third-order polynomial response was inadequate to describe this overall relationship but no further attempt was made to find a more complex model.

The effect of form of fertilizer was not significant ($P = 0{\cdot}111$) in the P model for PI species m^{-2} at the lowland meadow but was significant in the N model ($P = 0{\cdot}014$), with the overall linear response to the amount applied highly significant ($P < 0{\cdot}001$) in both models (P response shown in Fig. 3d). The N model predicted higher PI richness with inorganic treatments than with FYM, with an overall difference of 0·62 PI species m^{-2} (Fig. S1d).

These results suggest that treatments delivering up to about 10 kg P ha^{-1} $year^{-1}$ (equivalent to 11–12 t FYM ha^{-1} $year^{-1}$) were consistent with maintenance of species diversity at the upland meadow, with some indication that lower rates were beneficial. Only about 3 kg P ha^{-1} $year^{-1}$ applied as FYM (equivalent to about 4 t FYM ha^{-1} year^{-1}) or 5–6 kg P ha^{-1} year^{-1} as inorganic fertilizer was sustainable at the lowland site.

Soil chemical responses

Fertilizers raised both soil P and K noticeably by 2010 (Tables S2 and S3), but even with high rates of FYM application, concentrations of soil P at both meadows remained within the range typical of the relevant plant communities, whilst exchangeable K at both sites was within the higher range typical of improved grassland (Critchley *et al.* 2002b). No treatment effect was noticeable for soil organic matter, soil carbon or for total soil N.

Discussion

PLANT COMMUNITIES

The two sites together represent a wide range of unimproved meadows in the UK, Ireland and mainland Europe. In Britain, grasslands of the Geranio-sylvatici-Trisetetum association (MG3 according to the UK NVC, Rodwell 1992) are restricted to northern parts, but the broader Triseto-Polygonion alliance is widespread in montane and sub-montane regions of western and central Europe (Rodwell *et al.* 2007). Centaureo-Cynosuretum communities (MG5, Rodwell 1992) are much more widespread within the UK and the Republic of Ireland (Blackstock *et al.* 1999; Rodwell *et al.* 2007) and form part of the broader Cynosurion-cristati alliance characteristic of the less fertile, traditionally managed meadows in France and northern Spain (Rodwell *et al.* 2007).

The slightly higher initial species richness of the upland meadow compared to the lowland one was fairly typical of each grassland type (Critchley *et al.* 2002a), but by 2009, species richness in the lowland meadow vegetation receiving high rate FYM annually was more typical of agriculturally improved grasslands (Critchley *et al.* 2002a).

By 2010, both 12 and 24 t FYM ha^{-1} applied annually had raised weighted Ellenberg fertility scores in both meadows (Table S13) above the ideal range identified by Smith *et al.* (2003) for meadows equivalent to the upland site. Initial fertility scores (and NI% values, with which fertility scores were closely correlated) were considerably lower at the lowland site than at the upland site, whilst the response to fertilizer treatments was greater. At both sites, temporal trends and treatment effects in NI% were largely attributable to responses shown by the two NI species *Holcus lanatus* and *Poa trivialis*. Differences in the combined abundance of these species between the two plant communities represented here are typically slight (Rodwell 1992), suggesting that the large differences between the two study sites at the start of this study reflected historical differences in nutrient input rather innate differences in plant community type.

The damaging effects developing over time in response to quite modest nutrient inputs in this study are consistent with findings from studies covering a wide range of grasslands in Switzerland, Hungary and The Netherlands relating species richness to N input as an indicator of land-use intensity (Kleijn *et al.* 2009). In that study, the overall negative response was exponential, with the steepest decline over a relatively narrow range at the low end of the N scale, largely due to the disappearance of the scarcer (<1% of vegetation cover) species.

SOIL PROPERTIES AND NUTRIENT LIMITATION

Discrepancies between the N availability from FYM and the amounts actually applied in 'equivalent' inorganic treatments were much smaller than corresponding differences in P and were thus less likely to account for differences between fertilizer types in the their effects on total and PI species richness. Initial levels of extractable soil P at both sites were towards the low end of the range associated with maximal species richness in English grasslands (i.e. 4–15 mg L^{-1}, Critchley *et al.* 2002a), suggesting that soil P limitation was more likely than N limitation to be the main determinant of response to fertilizers in terms of botanical change. This justifies the main focus on P in the REML analyses, but does not exclude the possibility of colimitation by both nutrients, particularly at the lowland site where soil N reserves were relatively low. The treatments applied here provided no information on the latter aspect, however, since they included no variation in the proportions of N, P and K. Nevertheless, estimates of the amounts of N supplied by the most damaging treatment (24 t FYM ha^{-1} year^{-1}) were lower by about half – and those for P and K higher – than those contained in the lowest rates typically applied as commercial compound inorganic fertilizer or in fertilizer experiments (e.g. 25 kg N ha^{-1}, Mountford, Lakhani & Kirkham 1993; Kirkham, Mountford & Wilkins 1996). This suggests that whilst N use is a useful indicator of land-use intensity across a wide range of grasslands (Kleijn *et al.* 2009), it would be unwise to make decisions about ecologically sustainable fertilizer use in specific species-rich grasslands in terms of N input alone.

DIFFERENCES BETWEEN FYM AND INORGANIC FERTILIZERS

Whilst differences in effect between FYM and inorganic counterparts were largely accounted for by the lower amounts of P applied in the latter, remaining differentials might have diminished over a longer time following the revisions to the amounts of P (and N) applied in inorganic treatments. Furthermore, revised estimates of both N and P availability may not have fully accounted for accumulation of residues and for differences between FYM and inorganic fertilizers in this respect.

The implication that FYM is no less damaging to species-rich communities than inorganic fertilizers at equivalent levels of N, P and K application is very significant for the management of species-rich grassland in general. Organic manures may nevertheless be more desirable than equivalent inorganic fertilizers in that they provide extra food for soil invertebrates, including important prey items for birds (Marshall 1977; Vickery *et al.* 2001). However, any such benefits existing at these sites were not reflected in corresponding difference in soil carbon or organic matter content, even after 12 years and even at the lowland site where these values were generally low.

LIMING

Liming of all fertilizer treatments in 2005 recognized the traditional nature of occasional liming in hay meadows and also that no form of fertilizer is likely to be used in practice without periodic liming to maintain soil pH at levels sufficient to allow a yield response.

The difference between sites in response to liming in conjunction with annual FYM application probably reflects corresponding differences in past management, although, even after 12 years, there was only slight evidence of the plant community at the lowland meadow acclimatizing to this regime.

IMPLICATIONS FOR THE MANAGEMENT OF SEMI-NATURAL MEADOWS

Our results, from two long-term studies, show that the use of inorganic fertilizers supplying equivalent amounts of N, P and K to ecologically sustainable levels of FYM can be substituted for FYM in traditional meadow communities without additional negative impacts on species richness and composition. We also suggest that FYM inputs averaging <12 tonnes ha^{-1} year^{-1} or equivalent, applied annually or intermittently, are likely to be ecologically sustainable in species-rich meadows with a history of such inputs, with possible benefits of reducing inputs. In more oligotrophic meadows similar to that at the lowland site, inputs of <4 tonnes FYM ha^{-1} year^{-1} (or equivalent) should be considered maximum to preserve their botanical integrity. These levels are lower than the <6 tonnes FYM ha^{-1} year^{-1} previously suggested from earlier results (Kirkham *et al.* 2008). Our results also indicate that there is no effect of frequency of application *per se*, suggesting that decisions on whether to apply fertilizers annually at a low rate, or less frequently at a correspondingly higher rate, can be made on the basis of logistical or agronomic considerations alone. Finally, we show that occasional liming to raise soil pH to 6·0 is consistent with maintaining vegetation quality within meadows with a history of liming and may be introduced into other meadows as long fertilizer inputs are kept to a minimum. Overall our results show that knowledge of soil physical and chemical status and of past fertility management is important in deciding what level of fertilizer use might maintain or enhance the botanical diversity of species-rich grasslands.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Additional text on FYM analyses, liming and soil sampling.

Fig. S1. Modelled relationships between the mean amount of N applied per year (inorganic or FYM) and species richness (total and PI species).

Table S1. Soil chemical properties at experimental sites in 1999.

Table S2. Soil chemical properties in 2010 at the upland meadow.

Table S3. Soil chemical properties in 2010 at the lowland meadow.

Table S4. Positive and negative indicator species.

Tables S5–8. REML statistics for four key variables at the upland meadow 1999–2010.

Tables S9–12. ANOVA statistics for four key variables at the lowland meadow 1999–2010.

Table S13. Weighted Ellenberg fertility scores in 2010.