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Changes in plant species richness and productivity in response to decreased nitrogen inputs in grassland in southern England

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ABSTRACT

Biomass production and plant species diversity in grassland in southern England was monitored before and after a change from conventional to organic farming. Our 18-year study, part of the UK's Environmental Change Network long-term monitoring programme, showed that the cessation of artificial fertiliser use on grassland after conversion to organic farming resulted in a decrease in biomass production and an increase in plant species richness. Grassland productivity decreased immediately after fertiliser application ceased, and after two years the annual total biomass production had fallen by over 50%. In the subsequent decade, total annual grassland productivity did not change significantly, and yields reached 31–66% of the levels recorded pre-management change. Plant species richness that had remained stable during the first 5 years of our study under conventional farming, increased by 300% over the following 13 years under organic farm management. We suggest that the change in productivity is due to the altered composition of species within the plots. In the first few years after the change in farming practice, high yielding, nitrogen-loving plants were outcompeted by lower yielding grasses and forbs, and these species remained in the plots in the following years. This study shows that grassland can be converted from an environment lacking in plant species diversity to a relatively species-rich pasture within 10–15 years, simply by stopping or suspending nitrogen additions. We demonstrate that the trade-off for increasing species richness is a decrease in productivity. Grassland in the UK is often not only managed from a conservation perspective, but to also produce a profitable yield. By considering the species composition and encouraging specific beneficial species such as legumes, it may be possible to improve biomass productivity and reduce the trade-off.

1. Introduction

In 1939 the British parliament removed covenants protecting old grasslands from ploughing restrictions (Duffey et al., 1974). This, coupled with a desire for self-sufficiency, accentuated by the onset of the Second World War, marked the start of intensive agriculture in the United Kingdom. This intensification substantially increased between the 1940s, when two thirds of Britain's food was imported (Lloyd and Wibberley, 1977), and the 1980s when overproduction and subsequent detrimental effects on the environment were causing concern. By 1984 production of cereals was 10 million tonnes more than the population of the UK could utilise (Marren, 2002). Increased mechanisation and inorganic fertiliser use, as well as new strains of pasture species, including ryegrass and clover, created highly productive but species-poor swards.

In response to the loss of wildlife and degradation of landscape associated with this agricultural intensification (Ratcliffe, 1984; Hopkins et al., 2000), agri-environment schemes (AES) were introduced into the UK. Over the past 20 years three types of schemes have been introduced: Environmentally Sensitive Areas (ESAs), Countryside Stewardship (CSS) and Environmental Stewardship (ESS). The uptake of these schemes has substantially increased in the last 10 years: in 2005, 13% of the UK (Defra, 2007) was covered by AES, and by 2009 this had reached 66%, in excess of 6 million ha. In 2013 the area of land enrolled in entry level AES had risen to 7.4 million ha, compared to just under 2 million ha in 2005 (Defra, 2014).

Little is known about how the change from intensive to extensive farming methods affects land and biodiversity. Taylor and Morecroft (2009) followed and described plant and insect diversity trends at our study site in southern England for five years after the farmland was converted from conventional to organic agriculture. Those authors showed that above-ground biomass production in the pasture decreased rapidly after the management

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change, and that plant species richness had increased within two years. Although other authors have compared the effect of organic farming on plant species biodiversity and biomass production with that of conventional farming as paired comparisons, they do not detail – or have not assessed – the direct effect of the change in one area over time (Hole et al., 2005). There is some disagreement about the effect of nitrogen addition in agricultural systems. It has been shown that long-term nitrogen inputs increase standing crop biomass and decrease plant species richness, shifting plant composition to a few dominant species (Bakelaar and Odum, 1978; Tilman, 1987; Hunneke et al., 1990; Inouye and Tilman, 1995). However, other studies have shown that low species richness resulted in low above-ground biomass (Hector et al., 1999; Palmborg et al., 2005), although a recent re-analysis of one of these experiments has demonstrated that species richness and composition are likely to be of similar importance for productivity (Hector et al., 2011).

In this study, which forms part of the Environmental Change Network (ECN), a long-term monitoring programme started in 1992, we followed changes in vegetation in permanent pasture on the Wytham estate in Oxfordshire, UK, prior to and following the adoption of agri-environment measures under an ESA agreement. Building on the work of Taylor and Morecroft (2009), we assessed continued impacts on above-ground productivity and plant species richness for eight years beyond the original study, using data collected as part of additional ECN vegetation surveys in other parts of the study area.

2. Materials and methods

All experimental plots were situated on the Wytham estate (51.77 N, 1.34 W), as described by Taylor and Morecroft (2009). The estate, owned by Oxford University, is situated five km northwest of Oxford, and currently consists of approximately 670 ha of organic farmland, leased to FAI Farms Ltd, and 390 ha of woodland. The woodland consists of a variety of types, including ancient semi-natural woodland, secondary woodland and plantations. Until September 2001 the farmland was managed as a commercial mixed farm. In 2002 organic management was adopted, and by 2005 the farm had obtained organic status certification.

2.1. Plot description

The experimental plots in this study were of two types: grazing 'exclusion plots' in permanent pasture and 'vegetation plots' in grassland within the woods.

Ten exclusion plots were first set up on Lower Seeds permanent pasture (British grid reference: SP46720847) in 1996 to prevent grazing from livestock or wild herbivores (Fig. 1). Each exclusion plot measured 1.5 m × 2.5 m, was positioned randomly within a subplot (750 m^2) of a single main plot of 7500 m^2 and was covered with a wire mesh cage. The plots remained in the same position throughout the year and were relocated annually in March.

Six 10 m × 10 m vegetation plots were established between 1994 and 1998 in grassland within Wytham woods and in the surrounding farmland on the Wytham estate. Within these plots, ten 40 cm × 40 cm quadrats were randomly located and permanently marked with aluminium pegs, thus allowing the exact plots to be revisited annually.

Three of the six grassland plots, U1 (SP45710812), U2 (SP460008309), and U3 (SP46540762) were left 'unmanaged', other than for sporadic grazing and the ensuing natural fertilisation by sheep (Fig. 1). The remaining three plots, M1 (SP46710923), M2 (SP46570839) and M3 (SP46620770) were classified as 'managed' plots. They were all grazed by sheep and/or cattle, and were fertilised with nitrogen compounds prior to 2002; the nitrogen applications stopped in 2001. Between 1993 and 2000 (we do not have farm records for 2001) nitrogen was applied to the exclusion plots and the managed plots at a mean rate of 225 kg ha^{-1} $year^{-1}$. In addition, plot M3 was managed as historic grassland under the Higher Level Stewardship (HLS; Natural England, 2013) scheme.

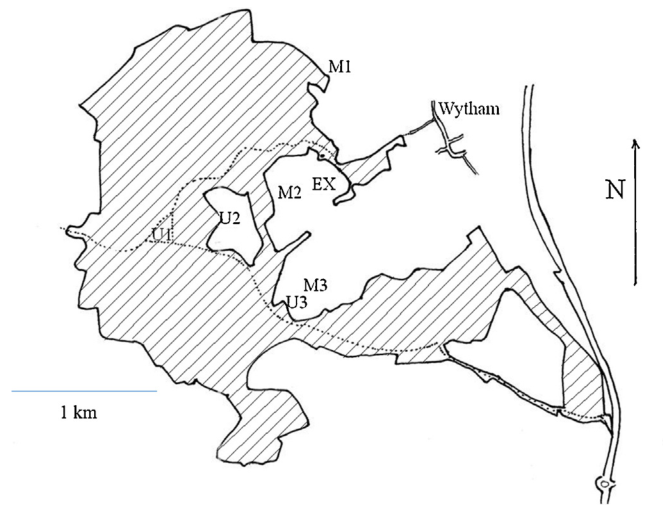

Fig. 1. Location of plots on the Wytham estate. U1–U3 are unmanaged plots on areas of rough grassland within the boundary of the woods. M1–M3 are managed plots on farmland and EX are the exclusion plots. Shaded area indicates woodland, clear areas indicate large areas of grassland.

2.2. Biomass production in permanent pasture

The pasture in the exclusion plots was cut four times during each year in May, July, September, and October, using a power scythe (Alpina, Global Garden Products Italy S.p.A. Veneto, Italy). A 10 cm strip was cut around the edge of each plot, raked off and discarded. The remaining uncut area was measured and the vegetation was then cut, collected and bagged for further processing and biomass calculation. The mesh cage was replaced in the exact spot it had been removed from (two opposite corners had been marked with aluminium tent pegs) and firmly pegged down to prevent accidental movement by grazing livestock. The vegetation removed from each plot was weighed and dried at 25 °C until the weight had stabilised and no more water was being lost. If the cut was large (>1 kg), a subsample of 100 g was used. Biomass was calculated and expressed as g m^{-2} $year^{-1}$.

2.3. Plant species diversity in the permanent pasture and grassland within Wytham woods

Between June and August, the six vegetation plots were surveyed annually as part of the ECN vegetation survey. Prior to the July cut the exclusion plots were also surveyed. The presence of all plants (graminoids, forbs, woody species and mosses) rooted in the soil within the small quadrats and in the exclusion plots were recorded. Species names follow Stace (2010) for vascular plants and Smith (2004) for bryophytes.

2.4. Data analysis

Data analysis was carried out in R version 3.2.2 (R Core Team, 2015). All analyses addressed the central hypothesis of whether a measured outcome (i.e. biomass, species richness or species frequency, depending on the experiment and model) exhibited a change significantly different from zero across the temporal shift from conventional to organic farming, here represented by the cessation of nitrogen fertilisation. The optimal structures of the individual models for each experiment and outcome type are discussed below; standard approaches to the selection of fixed effects, random effects, temporal auto-correlation and error structure distribution were used following Zuur et al. (2009).

2.4.1. Biomass production in permanent pasture

Biomass was log-transformed prior to inclusion in linear mixed models. Season of harvest and a categorical indicator for nitrogen application (i.e. during the period of annual nitrogen applications vs. the post-application period) were included as the covariates of interest. The inclusion of a random intercept term for each exclusion cage was preferred over a simple linear model (likelihood ratio test statistic = 3.91, P = 0.048), although the variance associated with the random intercept term was very low (0.005). Auto-correlation function (ACF) residual plots indicated the presence of strong temporal correlation. The inclusion of an auto-regressive moving average (ARMA) (1,0) error structure (Zuur et al., 2009) considerably reduced Akaike's Information Criterion (AIC) over a model that did not account for auto-correlation (1204.83 vs. 1228.44), and was included in the final model. The resulting linear mixed model was fitted using restricted estimate maximum likelihood using the R package 'nlme' (Pinheiro et al., 2015).

2.4.2. Species richness in permanent pasture

A generalised estimating equation (GEE; Højsgaard et al., 2006; Zuur et al., 2009) was used to assess the significance of the change in species richness across the temporal change in nitrogen application. The use of a GEE with a Poisson error structure improved the quantile–quantile (QQ) diagnostic plots of model residuals over a Gaussian linear model, and allowed for the inclusion of temporal auto-correlation (an auto-regressive series of order one was used; Zuur et al., 2009). Unlike the biomass model (Section 2.4.1 above), a random intercept for exclusion plot was not used; this was because model comparisons using AIC found little difference between simple and mixed models (with random intercept: 977.43; without: 975.43), and because of the fact that accounting for a Poisson error structure, temporal auto-correlation and random effects in a single model remains highly challenging (Bolker, 2015). Fisher's Exact Test was also used to compare the incidence of species pre- and post-farming management change; P values from these tests were adjusted for multiple comparisons using the false-discovery rate method of Benjamini and Hochberg (1995).

2.4.3. Species richness in managed and unmanaged vegetation plots

The significance of the effect of plot type and the presence or absence of nitrogen applications on species richness were assessed using a linear mixed model. AIC comparisons indicated that a model structure including nested random intercepts for quadrats within plots and a temporal auto-correlation structure (ARMA(1,0), as above) were both required. To achieve this, species richness was square-root transformed to enable the use of a linear mixed model (using the R package 'nlme'; Pinheiro et al., 2015). Comparison of QQ plots of linear model residuals using square-root transformed species richness with generalised linear models with a Poisson error structure on untransformed data showed similar improvements over linear models applied directly to count data. Similarly, Poisson generalised linear mixed models had much higher AIC values than linear mixed models accounting for temporal autocorrelation but using square-root transformed data (5073.04 vs. 1077.45); this suggested that accounting for temporal auto-correlation and spatial structure (the random effects) using transformed count data was preferred over modelling untransformed data where only one of the auto-correlation or random effects structures could be accounted for (Bolker, 2015). Fisher's Exact Test was again used to compare the incidence of species pre- and post-farming management change, as described above (Section 2.4.2).

3. Results

3.1. Biomass production in permanent pasture

An analysis of deviance of the final linear mixed model showed significant effects of season, nitrogen application and their interaction (Table 3). After nitrogen applications ceased in 2001 there was a rapid decline in biomass production (Fig. 2). This can be mostly attributed to the decrease in production in spring and early summer; autumn cut biomass showed no clear variation over years (Fig. 3). Seasonal variation, however, was dependent on the addition of nitrogen, with the autumnal decline in biomass being greater during the period of the applications (Table 3; Fig. SI1). In spring, productivity peaked in 1999 (527 g m^{-2}) and in summer productivity was greatest in 1997 (556 g m^{-2}). In the first year after the cessation of nitrogen fertilisation, spring biomass production decreased by 70%, and between 2002 and 2014 levels then fluctuated between 30 and 50% of the pre-2002 productivity. In summer, there was a lag in the response to the change in management: in 2002 biomass production was 523 g m^{-2}, 97% of

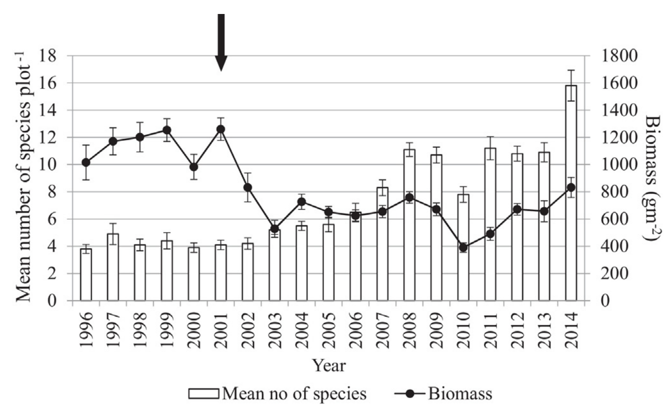

Fig. 2. Effect of ceasing the application of nitrogen fertiliser to permanent pasture on plant species richness and productivity. Columns show the mean number of species detected in 10 exclusion plots in July of each year. Solid black circles indicate total biomass production in each year. The black arrow indicates when the nitrogen applications stopped.

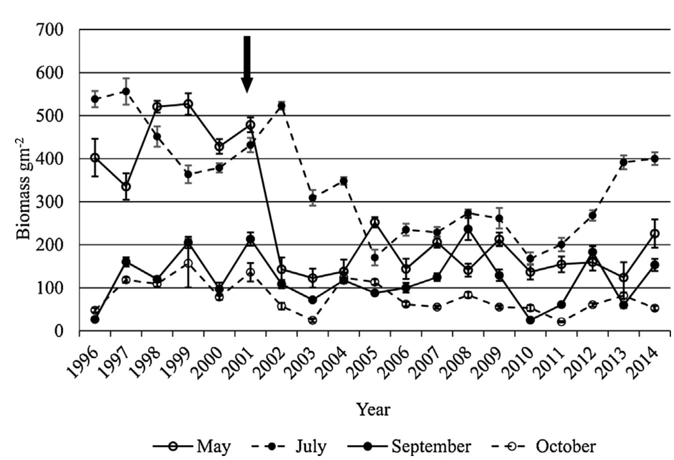

Fig. 3. Biomass production in permanent pasture in Wytham Woods between 1996 and 2014. The pasture was cut at four times during the growing season; spring (May), summer (July), and twice in autumn (September and October). The black arrow indicates when the nitrogen applications stopped.

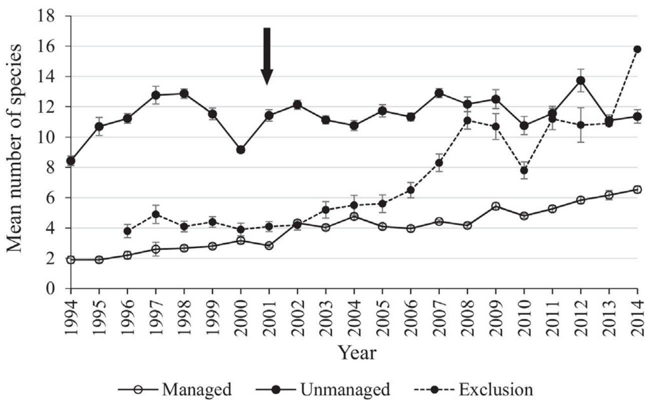

Fig. 4. Comparison of species diversity in exclusion plots vs. managed plots vs. unmanaged plots. The mean number of species rooted in the soil (graminoids, forbs, woody species and mosses) was calculated for the ten exclusion plots on the permanent pasture and also for the ten quadrats in each of the vegetation plots. The results for all the three managed plots were combined, as were the results for the unmanaged plots.

the maximum production pre 2002, but by 2005 production had decreased to a low of 170 g m^{-2}. Since 2010, and in contrast to the spring production, the summer yields have steadily increased, reaching 400 g m^{-2}. As expected, productivity was lower in autumn than in spring and summer.

3.2. Species composition in the permanent pasture

Annual monitoring of the vegetation in the exclusion plots in the permanent pasture showed that stopping the application of nitrogen fertilisers resulted in an increase in plant species diversity (Fig. 4; Fig. SI2). The mean number of species per exclusion plot rose from 4.2 before the change to organic farming to 8.7 after. In 2014 there was a mean of 15.8 species in each exclusion plot, with the maximum number of species detected in any one exclusion plot being 20. The mean number of species identified in each year between 1996 and 2001 was 10.3 and rose to 13.8 species between 2002 and 2006. Between 2007 and 2014, a mean of 24.9 species were detected. The greatest number of species was recorded in 2014, when 33 plant species were identified. An ANOVA of the final GEE model demonstrated a highly significant effect of a change in farming management on species richness (χ^2 = 19.2, d.f. = 1, P < 0.0001).

One effect of the cessation of nitrogen fertilisation was an alteration of the ratio of forbs to graminoids. Up to and including 2001, graminoids were most frequent in four out of six years, but after 2001 this was the case in only four out of 13 years. Comparison of the plant species detected between 1994–2001 and 2002–2014 (Table 2) showed that there were significant increases for *Agrostis stolonifera* (P < 0.001) *Arrhenatherum elatius* (P < 0.001), *Brachythecium rutabulum* (P < 0.05), *Bromus hordeaceus* (P < 0.001), *Cerastium fontanum* (P < 0.001), *Cynosurus cristatus* (P < 0.01), *Festuca rubra* (P < 0.01), *Geranium dissectum* (P < 0.001), *Medicago lupilina* (P < 0.001), *Phleum bertolonii* (P < 0.001), *Poa trivialis* (P < 0.001), *Ranunculus repens* (P < 0.001), *Taraxacum officinale* (P < 0.001) and *Trifolium repens* (P < 0.001). Not all species benefitted from the change in farming practice; *Alopecurus pratensis* (P < 0.05), *Cirsium arvense* (P < 0.01), *Poa annua* (P < 0.001), *Poa pratensis* (P < 0.05) and *Veronica agrestis* (P < 0.001) all suffered a significant decrease.

Table 1
Effect of the change from conventional to organic farming on species richness in the vegetation and exclusion plots. The number of species detected in all quadrats or cages were averaged over eight years (six years for exclusion plots) pre-change (1994–2001) and 13 years post-change (2002–2014). Plots prefixed U are unmanaged vegetation plots, whilst M indicates managed vegetation plots.

Plot	Mean number of species detected		Direction of change
	1994–2001	2002–2014	
U1	17.9	21.1	+
U2	29.2	28.9	–
U3	31.1	32.6	+
M1	4.1	11.5	+
M2	6.1	10.5	+
M3	6.5	10.1	+
Exclusion	10.2	19.2	+

3.3. Species composition in vegetation plots

3.3.1. Unmanaged plots

The unmanaged plots supported a more diverse range of plant species than the exclusion plots or the managed plots throughout the survey period (Table 1). A mean of 78.5 species was recorded in each plot between 1994 and 2014 and a mean of 53.3 species was identified in more than one year. In most years the number of forb species detected was greater than the number of graminoid species detected (Fig. 5), although the differences were largely insignificant. Comparison of each species detected prior to 2002 with records between 2002 and 2014 showed that 24 species increased significantly (Table 2) and 20 species decreased significantly. Of those that increased, 14 were forbs, five were graminoids, four were mosses and there was one woody species. For the forbs, the most significant increases in detection were for *Agrimonia eupatoria*, present in none of the quadrats prior to 2002 but 49% after (P < 0.01), *Cirsium eriophorum* (6.7–18.7%, P < 0.001), *Clinopodium vulgare* (0–12.8%, P < 0.001), *Convolvulus arvensis* (10–24%, P < 0.001), *Galium verum* (9–19.5%, P < 0.01), *Linum catharticum* (0–6%, P < 0.001) and *Veronica chamaedrys* (8–23.6%, P < 0.001). Of the four mosses that increased in detection, the greatest changes were for *B. rutabulum* (20–34.1%, P < 0.01) and *Kindbergia praelonga* (8.1–19.7%, P < 0.001). The detection of *Fraxinus excelsior* also increased significantly (4.7–26.4%, P < 0.001). Of the graminoid species that decreased, the most significant were *Luzula campestris* (21.9–10.8%, P < 0.01) and *P. pratensis* (15.2–4.6%, P < 0.001). Other species that decreased significantly were *Aphanes arvensis* (9–0.5%, P < 0.001), *C. fontanum* (31.9–15.9%, P < 0.001), *Galium aparine* (12.9–1.8%, P < 0.001), *G. dissectum* (21.4–7%, P < 0.001), *Sherardia arvensis* (10.9–2%, P < 0.001), *Rumex acetosa* (30.5–16.1%, P < 0.001), and *Veronica arvensis* (4.3–0%, P < 0.001).

3.3.2. Managed plots

Species richness in the managed vegetation plots within the Wytham estate was lower than in the unmanaged plots: a mean of 35.3 species was recorded in the plots between 1994 and 2014 (M1) or 1998 and 2014 (M2 and M3). Of these, a mean of 21.7 species were detected in more than one year. In the managed plots the majority of significant changes in species presence were increases in detection post-2001. Only four species significantly decreased in detection after this date: one forb, *A. arvensis* (2.7–0%, P < 0.001), and three graminoids: *Lolium perenne* (100–85.6%, P < 0.001), *P. annua* (37.3–5.1%, P < 0.001) and *P. pratensis* (12–0.7%, P < 0.001). In comparison, the detection of 31 species increased after the change from conventional to organic farming, 17 significantly so. Four species were not present until after 2001: *B. hordeaceus* (0–5.9%, P < 0.001), *P. bertolonii* (0–8.9%, P < 0.001), *R. repens* (0–26.2%, P < 0.01) and *T. repens* (0–25.6%, P < 0.001). The other species for which the change was significant were all graminoids and they increased between 6% and 41%. The greatest increase occurred in *Holcus lanatus* (22.7–63.3%, P < 0.001). For the other graminoids, the increase was smaller, but still significant, *Agrostis capillaris* (2–33.3%, P < 0.001), *A. stolonifera* (26.7–50%,

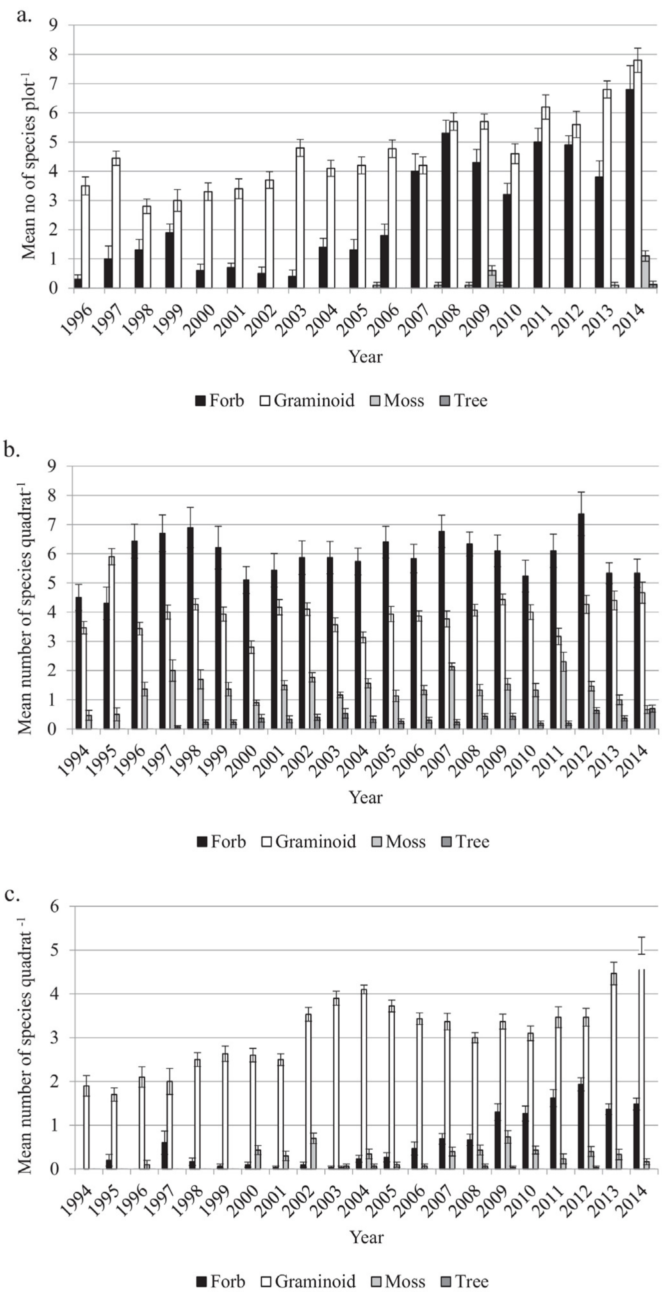

Fig. 5. Comparison of species diversity in the exclusion plots on permanent pasture with unmanaged and managed vegetation plots on the Wytham estate. The number of species representing four vegetation types: forbs (black columns); graminoids (white columns), mosses (light grey columns) and woody species (dark grey columns) was recorded and averaged across all plots (exclusion plots) or quadrats (vegetation plots). (a) Exclusion plots, (b) managed plots and (c) unmanaged plots.

P <0.001), *Dactylis glomerata* (10.7–42.8%, P < 0.001), *Phleum pratense* (0.7–8.7%, P < 0.001), and *P. trivialis* (8.7–28.7%, P < 0.001).

3.3.3. Comparison between managed and unmanaged plots

Not every plot contained all the species found throughout the survey, a reflection of the different soil and habitat types of the Wytham estate. We observed an increase in species diversity over the period of the survey in all managed plots and two out of three unmanaged plots (Table 1). An analysis of deviance of the final linear mixed model results is presented in Table 4. For the unmanaged plots, only small changes in the mean number of species detected pre- and post-management change were observed (Table 1). Changes in the managed plots and the exclusion cages in permanent pasture were more marked, with clear increases in average species richness (Table 1; Figs. SI2 and SI3).

The detection of species in the functional groups graminoids, forbs, mosses and woody species followed different patterns for different management systems. In the managed plots and exclusion cages graminoids were most common, but in the unmanaged plots the incidence of forbs and graminoids was similar, with forbs being more abundant in most years (Fig. 5). Woody species and mosses were more abundant in the unmanaged vegetation plots (Fig. 5) than in the managed plots. There was a gradual increase in the mean number of graminoids detected in the exclusion plots with 3.5 species in 1996 and 7.8 species in 2014 (Fig. 5a) and a similar steady increase in the managed plots, ranging from 1.9 in 1994 to 5.1 in 2014 (Fig. 5b). Forb species were absent or present at a low rate until the mid-2000s. In the exclusion plots less than two forb species per exclusion plot were detected until 2007, when there was a mean of 4 species per exclusion plot, and this continued to increase to 2014 when 6.8 species were detected. Similarly, forbs were almost absent from the managed plots until 2009 when a mean of 1.2 species per quadrat was detected. Until then less than one species per quadrat had been recorded. There were 35 plant species present in at least one of the managed or unmanaged plots or the exclusion plots and eight species (seven graminoids and one moss) were found in all plots. Of these eight species, four showed little or no difference frequency between the plot types, and four showed similarities between the managed plots and exclusion plots, but differences when compared with the unmanaged plots.

The presence of some species was affected by farming practice. *P. annua* was abundant in the managed plots and exclusion plots up to 2001 (between 90% and 100%, in the managed plots), but after the change to organic farming was implemented, the frequency of *P. annua* decreased to 50% (2004) and continued to decrease to 30% by 2014. Detection in the exclusion plots followed a similar pattern. In the unmanaged plots, however, *P. annua* was present in less than 20% of quadrats in all years between 1994 and 2014 and there was no change prior to and post 2001. The frequency of *B. hordeaceus* was also affected by the farming management change; this grass was detected in the unmanaged plots before 2002, but not in the exclusion plots or managed plots (except for one record in 1997). The presence of *B. hordeaceus* in unmanaged plots decreased after 2002, but increased in the managed plots and in the exclusion plots. Similarly, there were increases in the managed plots and exclusion cages for *H. lanatus* after 2002, but these increases were also seen in the unmanaged plots. Prior to 2002, *H. lanatus* was not detected in the exclusion cages, and was only detected in less than 10% of the managed plots in 1994.

4. Discussion

This study is one of a few long-term experiments demonstrating large shifts in plant community composition and grassland productivity following changes in nitrogen application and deposition, some of which have been reviewed by Dise and Stevens (2005) and Phoenix et al. (2012). In this study, high productivity before the move from a conventional to an organic system of farming corresponded to low species richness. After the change, the productivity of the pasture remained low although the species richness rapidly increased. However, in the last two years of this study productivity began to increase. In the UK, studies at Wardlow Hay Cop in Derbyshire showed that adding nitrogen contributed to the loss of species richness by decreasing forb cover and resulting in increases in graminoids (Morecroft et al., 1994; Carroll et al., 2003). Prior

Table 2
The effect of stopping nitrogen applications on species presence in vegetation plots. The percentage of plots in which the species was detected was averaged over eight years (six years for exclusion plots) pre change (1994–2001) and 13 years post change (2002–2014). These values were compared and are recorded below as an increase (+) or a decrease (–). Analyses were carried out using Fisher's exact test on actual plot presence/absence, asterisks indicate significant differences in the change: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. P values were corrected for multiple comparisons using the Benjamini–Hochberg method. Only species that were recorded in more than one plot, and that had a significant change in one of the plot types are included in the table.

Species	Change in unmanaged plots	Change in managed plots	Change in exclusion plots
Agrimonia eupatoria	+**	Not detected	+
Agrostis capillaris	–	+***	Not detected
Agrostis stolonifera	–*	+***	+***
Alopecurus pratensis	Not detected	+	–*
Aphanes arvensis	–***	–***	Not detected
Arrhenatherum elatius	+*	+*	+***
Brachythecium rutabulum	+**	+**	+*
Bromus hordeaceus	–	+**	+***
Calliergonella cuspidata	–**	Not detected	Not detected
Campyliadelphus chrysophyllus	–	Not detected	Not detected
Carex caryophyllea	+*	Not detected	Not detected
Cerastium fontanum	–***	+**	+***
Cirsium arvense	–	+*	–**
Cirsium eriophorum	+***	+	+
Clinopodium vulgare	+***	Not detected	Not detected
Conopodium majus	+	Not detected	Not detected
Convolvulus arvensis	+***	Not detected	–
Crepis capillaris	–**	+	+
Cynosurus cristatus	–	+**	+**
Dactylis glomerata	+*	+***	+
Festuca rubra	+**	+	+**
Fraxinus excelsior	+***	+	+
Galium aparine	–***	Not detected	+
Galium verum	+**	Not detected	Not detected
Geranium dissectum	–***	+	+***
Geranium molle	–**	Not detected	+
Glechoma hederacea	+**	Not detected	Not detected
Holcus lanatus	+*	+***	+
Hypericum hirsutum	+*	Not detected	Not detected
Kindbergia praelonga	+***	+	+
Linum catharticum	+***	+	+
Lolium multiflorum	–	+**	Not detected
Lolium perenne	–**	–***	+
Lotus corniculatus	+	+*	+
Luzula campestris	–**	Not detected	Not detected
Medicago lupulina	–	+	+***
Pastinaca sativa	–*	Not detected	+
Phleum bertolonii	+	+***	+***
Phleum pratense	+	+***	+
Pilosella officinarum agg.	+*	Not detected	+
Plagiomnium affine	–***	Not detected	Not detected
Poa annua	–**	–***	–***
Poa pratensis	–***	–***	–*
Poa trivialis	+	+***	+***
Polygala serpyllifolia	–**	–	Not detected
Potentilla reptans	+*	Not detected	Not detected
Prunella vulgaris	–*	Not detected	Not detected
Pseudoscleropodium purum	+*	Not detected	Not detected
Pteridium aquilinum	+*	Not detected	Not detected
Ranunculus repens	+*	+***	+***
Rhynchostegium confertum	–	+	Not detected
Rhytidiadelphus squarrosus	–**	+	Not detected
Rumex acetosa	–***	Not detected	Not detected
Schistidium apocarpum	+*	Not detected	Not detected
Sherardia arvensis	–***	Not detected	–
Taraxacum officinale agg.	+	+	+***
Trifolium repens	–	+***	+***
Trisetum flavescens	+	+	+
Urtica dioica	–	+	Not detected
Veronica agrestis	Not detected	Not detected	–***
Veronica arvensis	–***	–	Not detected
Veronica chamaedrys	+***	Not detected	+
Viola hirta	+**	Not detected	Not detected

to 2002, the dominant species in the exclusion plots at Wytham were fast growing pasture grasses: *L. perenne*, a palatable and nutritious grass (van Wijk et al., 1993), *P. annua*, *A. stolonifera* and *D. glomerata*. Although most of these productive grasses were still recorded in the exclusion plots after the change to organic farming, the composition of the plots had changed to include a greater number of forbs and low-yielding grass species. Species that increased in frequency included *C. cristatus*, which has a low yield due to the high stem to leaf ratio; those that would not withstand heavy grazing e.g. *A. elatius*; and those regarded as grass weeds with

Table 3
Analysis of deviance results of the linear mixed model for log-transformed biomass data from exclusion cages.

Model term	Numerator d.f.	Denominator d.f.	*F* value	*P* value
Intercept	1	711	26,766.22	<0.0001
Season	2	711	455.05	<0.0001
N application	1	711	128.98	<0.0001
Season × N application	2	711	16.91	<0.0001

Table 4
Analysis of deviance results of the linear mixed model for square-root transformed species richness in managed and unmanaged vegetation plots.

Model term	Numerator d.f.	Denominator d.f.	*F* value	*P* value
Intercept	1	1078	296.45	<0.0001
N application	1	1078	99.30	<0.0001
Plot type	1	4	17.47	0.014
N application × plot type	1	1078	32.07	<0.0001

low yield or which are typically unpalatable to stock, e.g. *P. trivialis* and *H. lanatus*. Our results suggest that the composition of species is more important for determining productivity than simply the number of species present. Marquard et al. (2009) found that in a six year biodiversity experiment in Germany, the increase in biomass was positively correlated with species richness, and that doubling the number of species increased community biomass by 91 g m^2. They also showed that some groups of species had different effects on productivity, particularly legumes, which had a positive effect on community biomass. At Wytham, one legume species, *T. repens*, was present in only one plot prior to 2001 (in 1997 and 1999). However, the frequency of many legumes has increased since 2008 (*L. corniculatus*, *M. lupilina*, *Trifolium dubium*, *T. repens*, *Trifolium pratense*, *Vicia sativa* and *Vicia tetrasperma*) with all but *V. tetrasperma* being detected in 2014. In the German study, grasses had only a small effect on community biomass, and Marquard et al. (2009) suggested that these graminoids were being outcompeted by tall forbs and legumes. However, our study shows that when nitrogen inputs were high, and grasses more frequent than forbs, mosses and woody species, the productivity was also high. If legumes generally make significant contributions to biomass production (Marquard et al., 2009), this may explain why we observed a 100% increase in spring productivity in our exclusion plots in 2014 compared with 2005. Marquard et al. (2009) also suggested that tall herbs made a significant contribution to productivity; however, in our studies we recorded few tall herbs in our plots.

Other studies have also shown that the composition of the grassland plant community can affect above-ground biomass, and that increased species richness can result in increased biomass productivity (Tilman et al., 1996, 2001; Hector et al., 1999; Bullock et al., 2001), and provides a reasonable explanation for the recent yield increases at Wytham. A meta-analysis of 44 biomass and species composition experiments showed that mixtures of species produce on average 1.7 times more biomass than species monocultures (Cardinale et al., 2007). These results suggest that the precise composition of a community, and how the species with it interact and compete with each other for available resources, is also important. Cardinale et al. (2007) also found that as the duration of the study increased, so did the probability that species mixtures would yield more than their most productive species alone. This may be another reason why the productivity of our exclusion plots increased in the last two years of the study as species richness increased. It may, therefore, take several more years before maximum productivity in our permanent pasture is reached, especially if the diversity of species is increasing.

All of our managed plots showed an increase in species richness after 2001, albeit to different extents depending on the location of the plot, but in the unmanaged plots there was no such identifiable change in species richness. After the cessation of nitrogen inputs, it took two years for species richness to increase in the managed plots, and seven years for the average number of species detected in the plots to double. In contrast, after five years of adding nitrogen fertiliser to a sandy grassland in North-eastern China, Shi et al. (2014) found that three years post-fertilisation was long enough for species richness to begin to show signs of recovery.

Rundlöf et al. (2010) showed that, in Sweden, organic farming resulted in a significantly greater abundance of forbs than in conventional farming. Other farm surveys have shown that increasing the rate of nitrogen fertiliser increases the proportion of *Lolium* spp. at the expense of *Agrostis* spp. (Jones and Hayes, 1999; Hopkins et al., 1990), white clover (*T. repens*) and forbs (Hopkins et al., 1990). As mentioned above, we showed that clover and other legumes benefitted greatly from the cessation of nitrogen inputs. The nitrogen-loving grasses were still detected in a high percentage of the plots, but we did not measure percentage cover so we cannot comment on their abundance within exclusion plots. In the USA, Foster and Gross (1998) found that escalating nitrogen inputs increased the dominance of one native grass species (*Andropogon gerardi*), thus preventing establishment of subordinate forb species and reducing overall species diversity. At Wytham, we also observed a change in species composition: some nitrogen loving species such as *P. annua* disappeared from the exclusion plots, and there was an increase in generalist grasses such as *Agrostis* spp. and *Phleum* spp. Mountford et al. (1996) observed that at Tadham Moor (Somerset, England) the effects of fertiliser inputs were reversible: low levels (25 kg ha^{-1}) of fertiliser resulted in increased *H. lanatus* and *L. perenne*, and significantly reduced species diversity within six years. However, once nitrogen additions had stopped, *H. lanatus* and *L. perenne* declined in the plots and their place was taken by *A. capillaris* and *C. cristatus*. Hopkins et al. (2000) corroborated these findings, also suggesting that these botanical changes due to increased nitrogen were to some extent temporary if nitrogen additions ceased. Whilst this was the case for the managed plots at Wytham, we found that *H. lanatus* did not appear in the exclusion plots until the nitrogen applications had stopped, and that *H. lanatus* then increased rather than decreased. In a study in North America examining the effect of cessation of nitrogen fertilisation on species re-colonisation of grassland over a 30 year period, Isbell et al. (2013) reported that, subsequent to 10 years of high nitrogen input, it took 20 years to return for plots to return to control levels of species diversity. These studies suggest that the species diversity in our plots may continue to rise for several more years. In a study in the Netherlands, Smits et al. (2008) found that maximum species richness was reached 10–15 years after the cessation of fertiliser application and that, after 20 years, species richness then began to decrease. These authors attributed this decrease to two reasons: either plant species had reacted to the changing levels in the available nutrients, or atmospheric nitrogen deposition and soil eutrophication had altered the levels of available nitrogen. Mountford et al. (1996) estimated that it would take 9 years for grassland that had received nitrogen inputs of 200 kg ha^{-1} to revert back to its plant species composition prior to fertilisation. In our study, all managed plots, including the exclusion plots, were subjected to over 200 kg ha^{-1} nitrogen each year between 1992 and 2001. Over the subsequent 13 years, the species diversity in the managed plots (again including the exclusion plots) increased. In the exclusion plots, by 2014, there were more species present than in the unmanaged plots, but in the other managed plots species diversity was considerably lower.

In our study, 20 years of vegetation monitoring has provided valuable and rare data on the long-term effect of the cessation of nitrogen inputs on grassland. We have demonstrated that moving to an organic farm management system is beneficial to plant species diversity in permanent grassland, resulting in a greater number of both grasses and forbs without introducing seed mixtures. Although not investigated in this study, it is likely that the increase in plant species diversity will also impact other organisms, especially invertebrates (Duffey et al., 1974). We showed that biomass production initially decreased after artificial fertiliser inputs had ended, but that as species diversity subsequently increased, biomass also began to show signs of recovery, possibly due to the increase in plants such as legumes that not only add to the quality of the herbage but can also increase soil fertility (Brockwell et al., 1995). By not re-sowing the grassland and using minimal management, i.e. occasional grazing and/or cutting, grassland can recolonise with species local to the area. This work suggests that in the medium term, biodiversity can be improved without having to spend time and money improving soil quality, or habitat features, or investing effort to sow imported seed, although it should be noted that this may depend on the history and landscape-context of the grassland in question. Increasing biodiversity by this approach acknowledges that there may be a temporary reduction in grassland productivity. Grassland monitoring at Wytham as part of the UK ECN programme will continue, and we hope ultimately to be able to determine when maximum species diversity is reached, and whether biomass productivity will eventually reach levels recorded before the conversion to organic farming.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2015.12.024.

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