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Park Grass

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trees, dominated by oak and ash. An understory of holly has become increasingly dense since the 1960s. Because the soil has become so acid, there are few ground cover species.

On both sites, much C has been sequestered in trees and soil since cultivation ceased in the 1880s (Poulton et al., 2003). By the end of the 20th Century, Geescroft had gained, on average, 2.00 t C ha⁻¹ yr⁻¹ (0.38 t in litter and soil to a depth of 69cm, plus an estimated 1.62 t in trees, including their roots); corresponding gains of N were 22.2 kg N ha⁻¹ yr⁻¹ (15.2 kg in soil, plus 6.9 kg in trees). Broadbalk has gained 3.39 t C ha⁻¹ yr⁻¹ (0.54 t in soil, plus an estimated 2.85 t in trees), 49.6 kg N ha⁻¹ yr⁻¹ (36.8 kg in soil, plus 12.8 kg in trees). Much of the N required for plant growth will have come from inputs in rain and dry deposition. The faster accumulation of C and N in the wooded part of Broadbalk compared to Geescroft is probably because, as it is relatively narrow, there is a large edge effect and greater light interception per unit area, perhaps more scavenging of atmospheric N, and thus more growth. However, additional atmospheric N could have come from nearby covered yards in which bullocks were housed during the winter.

Park Grass



Park Grass, 1941

Park Grass is the oldest experiment on permanent grassland in the world. Started by Lawes and Gilbert in 1856, its original purpose was to investigate ways of improving the yield of hay by the application of inorganic fertilisers or organic manures (Plan 2 and Table 3).

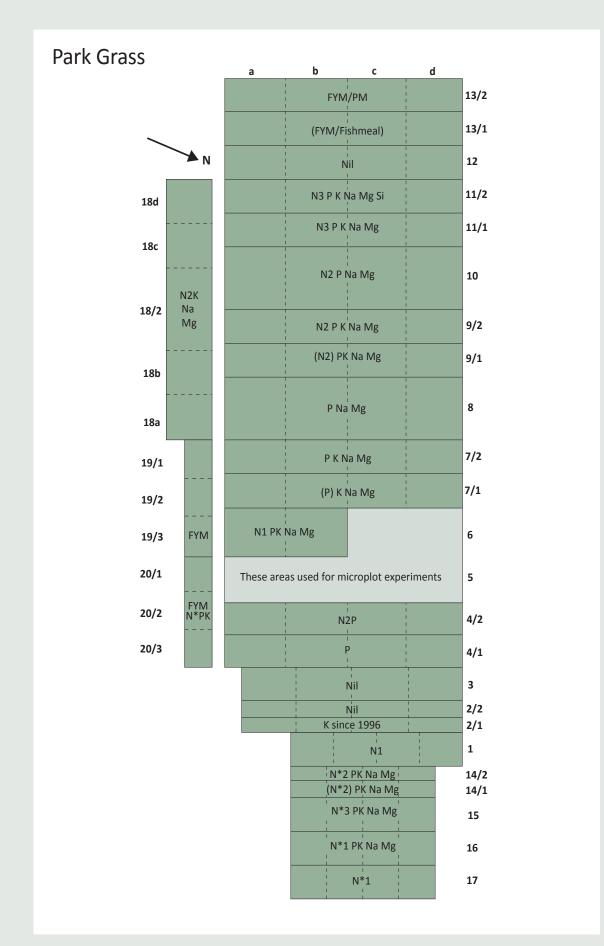
Within 2-3 years it became clear that these treatments were having a dramatic effect on the species composition of what had been a uniform sward comprising about 50 species. The continuing effects on species diversity and on soil function of the original treatments, together with later tests of liming and interactions with atmospheric inputs and climate change, has meant that Park Grass has become increasingly important to ecologists, environmentalists and soil scientists (Silvertown *et al.*, 2006). It is a key Rothamsted site within the UK Environmental Change Network (see later).

The experiment was established on *c*. 2.8 ha of parkland that had been in permanent pasture for at least 100 years. The uniformity of the site was assessed in the five years prior to 1856. Treatments imposed in 1856 and subsequently included controls (Nil - no fertiliser or manure),

and various combinations of P, K, Mg, Na, with N applied as either sodium nitrate or ammonium salts (Table 3). FYM was applied to two plots but was discontinued after eight years because, when applied annually to the surface in large amounts, it had adverse effects on the sward. FYM, applied every four years, was re-introduced on three plots in 1905.

The plots are cut in mid-June and made into hay. For 19 years the re-growth was grazed by sheep penned on individual plots but since 1875 a second cut, usually carted green, has been taken. The plots were originally cut by scythe, then by horse-drawn and then tractor-drawn mowers. Yields were originally estimated by weighing the produce, either of hay (1st harvest) or green crop (2nd harvest), and dry matter determined from the whole plot. Since 1960, yields of dry matter have been estimated from strips cut with a forage harvester. However, for the first cut the remainder of the plot is still mown and made into hay, thus continuing earlier management and ensuring return of seed. For the second cut the whole plot is cut with a forage harvester.

Park Grass probably never received the large applications of chalk that were often applied to arable fields in this part of England. The soil (0-23cm) on Park Grass probably had a pH (in water) of about 5.5 when the experiment began. A small amount of chalk was applied to all plots during tests in the 1880s and 1890s. A regular test of liming was started in 1903 when most plots were divided in two and 4 t ha⁻¹ CaCO₂ applied every four years to one half. However, on those plots receiving the largest amounts of ammonium sulphate this was not enough to stop the soil becoming progressively more acid, making it difficult to disentangle the effects of N from those of acidity. It was decided to extend the pH range on each treatment and, in 1965, most plots were divided into four: sub-plots "a" and "b" on the previously limed halves and sub-plots "c" and "d" on the previously unlimed halves. Sub-plots "a", "b" and "c" now receive different amounts of chalk, when necessary, to achieve and/or maintain soil (0-23cm) at pH 7, 6 and 5, respectively. Sub-plot "d" receives no lime and its pH reflects inputs from the various treatments and the atmosphere. Soils on the unlimed sub-plots of the Nil treatments are now at c. pH 5.0 whilst soils receiving 96 kg N ha⁻¹ as ammonium sulphate or sodium nitrate are at pH 3.4 and 5.9, respectively. For the latter two treatments, between 1965 and 2015, 74 and 22 t ha⁻¹ CaCO₂, respectively, were required to increase the soil pH and maintain it at pH 7.



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Table 3 Park Grass fertiliser and organic manure treatments.

Treatments (per hectare per year unless indicated)

Nitrogen (applied in spring)

N1, N2, N3	48, 96, 144 kg N as ammonium sulphate
N*1, N*2, N*3	48, 96, 144 kg N as sodium nitrate
(N2) (N*2)	last applied 1989

Minerals (applied in winter)

	 ,
Р	17 kg P as triple superphosphate since 2017, previously 35 kg P
Κ	225 kg K as potassium sulphate
Na	15 kg Na as sodium sulphate
Mg	10 kg Mg as magnesium sulphate
Si	450 kg of sodium silicate
Plot 20	30 kg N*, 15 kg P, 45 kg K in years when FYM is not applied

In 2013, plot 7 was divided into 7/1 and 7/2; P applications on 7/1 stopped Since 2013, plot 15 has also received N*3 (previously PKNaMg but no N)

Organics (applied every fourth year)

FYM35 t ha⁻¹ farmyard manure supplying c.240 kg N, 45 kg P, 350 kg K, 25 kg Na, 25 kg Mg, 40 kg S, 135 kg CaPMPelleted poultry manure (replaced fishmeal in 2003) supplying c.65 kgN

On plot 13/2 FYM and PM (previously fishmeal) are applied in a 4-year cycle i.e.:

FYM in 2017, 2013, 2009, 2005 etc. PM in 2015, 2011, 2007, 2003, fishmeal in 1999, 1995 1991 etc.

(FYM/Fishmeal) FYM and fishmeal last applied in 1993 and 1995 respectively

Lime (applied every third year)

Ground chalk applied as necessary to maintain soil (0-23 cm) at pH 7, 6 and 5 on sub-plots "a", "b" and "c". Sub-plot "d" does not receive any chalk

In 1990, plots 9 and 14, which received PKNaMg and N as either ammonium sulphate or sodium nitrate respectively, were divided so that the effects of withholding N from one half of all the sub-plots could be assessed. Similarly, plot 13, which received FYM and fishmeal (now poultry manure), was divided, and, since 1997, FYM and fishmeal has been withheld from one half. In 1996, plot 2, a long-term Nil treatment, was divided and K has been applied to one half each year to give a "K only" treatment. In 2013, plot 7 was divided in two to test the effects of withholding P on herbage production and botanical diversity. The effects have been negligible so far, almost certainly because of the large amounts of available P that had built up in the soils from past inputs; in 2014 available P on plots receiving P fertiliser was 60-290 mg plant-



Park Grass, plots 11/2d (left) and 12d (right)

available (Olsen)P kg⁻¹. Consequently, in 2016, the P application to these plots was decreased from 35 to 17 kg P ha⁻¹, so that it more closely matches P offtakes. Since 2013, plot 15 has received sodium nitrate at 144 kg N ha⁻¹, in

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addition to PKNaMg, to provide a comparison with plot 11, which receives the same rate of N as ammonium sulphate.

Yields of total dry matter (both harvests) for 2012-16 are shown in Table 4. The largest yields were on limed sub-plots given PKNaMg and 144 kg N ha⁻¹ (11/1 and 11/2). Yields with 96 kg N ha⁻¹ as either ammonium or nitrate (and PKNaMg) are similar (9/2 and 14/2); where P or K are not applied yields are less (18, 4/2 and 10). Similarly, yields on plots given N only (1 and 17) are no better than on the Nil plots (3, 12 and 2/2) because lack of P and K limits yield. On soil receiving PKNaMg but no N fertiliser (7/2), yields are as good as those on plots receiving PKNaMg plus 96 kg N ha⁻¹ (plots 9/2 and 14/2) because of the large proportion of legumes in the sward (Table 4). Where no lime is applied legumes are less common and yields

		Sub-plot										
Plot	Treatment ⁽¹⁾	а	b	с	d							
No nitrogen group	•••••••••••••••••••••••••••••••••••••••		••••••	••••••	••••••							
3	Nil	3.3	3.6	1.8	2.7							
12	Nil	4.0	3.3	2.7	2.6							
2/2	Nil	3.8	3.7	2.6	2.9							
2/1	К	3.4	3.8	2.3	2.0							
4/1	Р	4.8	5.2	4.1	3.9							
8	P Na Mg	4.6	4.7	4.1	4.3							
7/1 ⁽²⁾	(P) K Na Mg	6.5	7.2	6.6	4.4							
7/2 ⁽²⁾	P K Na Mg	6.7	6.8	6.6	5.0							
Ammonium N group												
1	N1	3.6	3.1	2.3	1.7							
- 18	N2 K Na Mg	3.9	3.9	3.6	2.4							
4/2	N2 P	3.9	4.3	4.4	2.8							
10	N2 P Na Mg	4.8	5.0	5.3	3.6							
6	N1 P K Na Mg	6.9	7.2	-	-							
9/1	(N2) P K Na Mg	7.0	7.3	5.8	1.7							
9/2	N2 P K Na Mg	7.1	7.4	6.3	5.1							
11/1	N3 P K Na Mg	8.0	7.2	7.0	6.0							
11/2	N3 P K Na Mg Si	8.6	8.2	7.5	7.0							
Nitrate N group		••••••	••••••	••••••	•••••							
17	N*1	3.6	3.9	2.9	3.3							
16	N*1 P K Na Mg	6.9	7.0	6.9	5.6							
14/1	(N*2) P K Na Mg	6.8	7.1	7.0	6.9							
14/2	N*2 P K Na Mg	6.5	6.6	6.7	6.7							
15 ⁽³⁾	N*3 P K Na Mg	7.3	7.4	7.3	7.1							
FYM group	••••••			•••••								
13/1	(FYM/fishmeal)	5.6	5.6	4.9	4.5							
13/2	FYM/PM	5.8	6.9	6.9	6.4							
	••••••	/1	/2	/3								
19 ⁽⁴⁾	FYM	•	7.2	6.3								
20 ⁽⁴⁾	FYM FYM/N* P K	6.9 7.1	7.2	6.8								
2017	•	7.1		0.0								

 $^{\scriptscriptstyle (1)}$ See Table 3 for details

⁽²⁾ Plot 7 split in 2013 and P withheld from 7/1; yields given for 7/1 are for 2013-16

⁽³⁾ N*3 applied since 2013 (yields given are for 2013-16)

⁽⁴⁾ Plots 19 and 20 are not part of the liming scheme

are smaller. For all treatments, yields on unlimed sub-plots are less than those on soils maintained at pH 6 or above. However, even on the very acid soils (pH 3.4 - 3.7) dominated by one or two species, mean yields can still be as large as 6-7 t ha⁻¹ (*e.g.* "d" sub-plots of 11/1 and 11/2).

Botanical composition

Vegetation surveys have been carried out on Park Grass on more than 30 occasions since the experiment began. The most recent, comprehensive surveys of botanical composition, made just before the first cut, were done annually from 1991 to 2000 and from 2010 to 2012. Table 5 shows soil pH and those species comprising 5% or more of the above ground biomass, and the total number of species identified on each subplot (selected treatments, mean 2010-2012). The striking contrasts between the plots, in botanical diversity and composition, are a result of complex interactions between fertiliser and manure treatments and pH. Without exception, all the original treatments imposed at the start of the experiment resulted in a decline in species number; the fertilisers have acted on the community by selecting out species that are poorly adapted to those treatments. When the effect of increasing soil fertility is analysed separately from the effect of pH, the steepest declines in species richness have been observed on plots that receive both inorganic N and P in combination.

The most diverse flora, including many broad-leaved species, is on the Nil plots (plots 3, 2/2 and 12), with about 35-42 species in total. These swards are probably the nearest approximations to the species composition of the whole field in 1856, although gradual impoverishment of the plant nutrients soon caused decreases in perennial ryegrass (*Lolium perenne*) and Yorkshire fog (*Holcus lanatus*) and later increases in common bent (*Agrostis* *capillaris*), red fescue (*Festuca rubra*), rough hawkbit (*Leontodon hispidus*) and common knapweed (*Centaurea nigra*). Species characteristic of poor land *e.g.* quaking grass (*Briza media*) and cowslip (*Primula veris*) are also present in small amounts, on these plots. Lime alone does not greatly alter the absence/ presence of individual species but it decreases the contribution of common bent and red fescue, and increases that of some broadleaved species.

Applying N as ammonium sulphate or as sodium nitrate has resulted in the most spectacular contrasts. In the absence of applied chalk, soil pH on the "d" sub-plots ranges from 4.1 to 3.6 where ammonium sulphate has been applied and from 5.4 to 6.0 with sodium nitrate. The effect of soil acidification on the total number of species in the sward is dramatic; 1-4 species with ammonium sulphate, but 22-35 with sodium nitrate (Table 5). Grasses are dominant on the "d" sub-plots, where the soil pH ranges from 4.0 to 3.6. Species that dominate on these plots, such as sweet vernal grass (Anthoxanthum odoratum), are restricted to those able to tolerate the increased concentration of aluminium ions in the soil associated with low pH. Figure 6 summarises, for three contrasting treatments,



Sorting herbage samples from Park Grass, 2010

Percentage of dry matter (Species names are listed below) No. o						No. of																	
Treatment ⁽¹⁾	Plot	Soil pH in 2011	AC	АР															LH	PL	RA	SM	species observed
Nil	3a b c d	7.2 6.3 5.2 5.3	+ 5 10 15	+ + - -	+ + + +	+ + - +	5 5 5 5 5	+ + + +	10 10 30 25	+ 5 +	+ + + +	15 10 5 +	5 5 5 +	+ + - +	+ 5 + 5	10 5 5 5	- - + -	- - + +	10 15 20 10	5 10 5 5	+ + + +	10 10 + -	37 35 37 35
Nil	2/2b d	6.2 5.1	+ 15	+ -	+ +	+ + +	5 5	+ + +	10 30	5 +	+ -	10 +	5 +	+ -	+ 5	10 5	+ -	- +	20 5	5 10	+ + +	5 -	42 35
K (since 1996)	2/1b d	6.0 4.8	+ 20	+ -	+ +	+ -	10 +	+ + +	10 30	5 +	+ -	5	15 +	+ -	5 5	5 5	+ -	- 5	25 10	5 5	+ + +	+ -	39 28
PKNaMg	7b d	6.2 4.9	+ 10	+ +	+ 5	25 +	-	5 +	+ 15	+ 5	5 5	- +	25 15	+ 5	+ 5	+ 10	5 +	- +	- 5	10 20	+ +	- -	29 33
(FYM/ Fishmeal)	13/1b d	6.2 4.8	10 20		5 5	15 -	-	5 +	5 10	5 5	10 +	+ +	10 15	- +	+ 10	+ +	+ -	+ 10	5 5	10 10	5 5	- -	33 33
FYM/PM	13/2b d	6.1 5.0	5 30	10 5	+ 10	20 5	-	10 +	5 10	10 10	15 -	- -	+ 10	- -	- 10	- +	5 +	- -	+ 5	5 5	5 5	- -	33 30
N*1	17b d	6.3 5.7	5 5	+ +	+ 5	+ + +	20 10	5 +	5 5	5 5	- -	- -	++++	+ -	5 +	5 5	+ + +	- -	25 35	10 10	+ + +	+ -	36 35
(N*2) PKNaMg	14/1a b c d	6.9 6.0 5.3 5.4	+ + 5 10	+ 5 + 5	+ + 5 5	15 10 5 10	- - - -	5 + 10 5	5 5 5 +	+ + 5 5	+ 15 10 5	+ - + -	25 25 25 25 20	15 + 5 10	+ + - +	+ + + +	15 10 5 +	- - - -	- - + +	5 10 10 10	5 5 5 5 5	- - - +	29 29 31 26
N*2 PKNaMg	14/2a b c d	7.0 6.2 5.9 6.0	- + + +	25	+ + + +	35 25 25 15	- - -	5 + 5 5	5 5 5 5	10 10 10 10	+ + + -	- - - -	5 5 5 +	+ + + +	+ - -	- + + -	20 10 5 5	- - -	- + + -	+ 5 5 5 5	5 5 10 10	- - - -	25 28 25 22
N1	1b d	6.3 4.0	5 65	- -	+ 30	+ -	30 -	5 -	5 +	+ -	- - -	+ -	+ -	- -	+ -	5 -	- -	- -	25 +	10 -	+ -	- -	28 5
N2P NaMg	10b d	6.3 3.7	10 5	+ -	10 90	+ + +	-	-	40 -	20 5	- -	+ -	-	- -	- -	- -	- -	-	-	15 -	+ -	- -	18 4
(N2) PKNaMg	9/1a b c d	7.1 6.4 5.2 4.1	+ + 5 45	+ 5 + -	+ + + 45	10 10 5 -	- - -	5 + + -	+ + 10 +	+ + 5 +	15 5 5 -	- + + -	20 45 25 +	+ + 5 -	+ + 5 -	- 5 10 -	5 + + -	- - -	+ + + -	10 15 10 +	+ + + -	- - -	30 34 31 11
N2 PKNaMg	9/2a b c d	7.1 6.2 5.1 3.7	- + 20 +	5	+ + 55	35 40 10 -	- - - -	10 5 5 -	+ + 30 -	5 5 10 40	10 5 5 -	- - + -	15 15 5 -	- - -	- - -	- - - -	5 5 + -	- - -	- - -	+ 5 + -	+ + + -	- - -	23 24 28 3
N3 PKNaMg	11/1b d	6.4 3.6	-	20 -	+ -	30 -	-	20 -	+ -	10 100		- -	+ -	- -	- -	- -	5	- -	-	+ -	+ -	- -	17 1
N3 PKNaMgSi	11/2b d	6.1 3.6	- 5	20	+ +	45 +	-	15 -	-	5 95	-	-	+ -	-	-	+ -	5	-	- -	+ -	+ -	- -	17 4

Table 5. Park Grass; species comprising at least 5% of herbage, mean 2010-2012; and total number of species observed

(1) See Table 3 for treatment details.

Data are from surveys immediately before hay harvest; mean 2010-2012 rounded to the nearest 5% of dry matter (selected plots only). Note; +, species present at less than 5%; -, species not present on that plot.

Species that do not occur at 10%, or more, on any one plot are not shown.

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Grasses	Agrostis capillaris Alopecurus pratensis Anthoxanthum odoratum Arrenatherum elatius Briza media Dactylis glomerata Festuca rubra Holcus lanatus	Common Bent Meadow Foxtail Sweet Vernal Grass False Oat Grass Quaking Grass Cock's-foot Red Fescue Yorkshire Fog
Forbs	Achillea millefolium Centaura nigra Heracleum sphondylium Hypochaeris radicata Leontodon hispidus Plantago lanceolata Ranunculus acris Sanguisorba minor	Yarrow Common Knapweed Hogweed Cat's-ear Rough Hawkbit Ribwort Plantain Meadow Buttercup Salad Burnet
Legumes	Lathyrus pratensis Lotus corniculatus Trifolium pratense Trifolium repens	Meadow Vetchling Common Bird's-foot-trefoil Red Clover White Clover

effects over time on the numbers of species comprising 1%, or more, of the above-ground biomass. Even on the Nil plots, the number of species has decreased since the start of the experiment, possibly as a consequence of atmospheric inputs and/or changes in the management of the sward. Applying either form of N decreased species number further in the absence of chalk, much more so with ammonium sulphate than with sodium nitrate. Raising soil pH, by adding chalk, has had bigger effects on the Nil and ammonium sulphate treatments than on those given sodium nitrate.

Since 2000 an increase in legumes, as a percentage of herbage dry matter, has been observed on plots 9/1 and 14/1, where fertiliser N has been withheld since 1989, and on other treatments (Table 5). Over the same period a marked decrease in atmospheric N deposition has been observed, indicating that grassland species diversity can recover following a decrease in atmospheric pollution

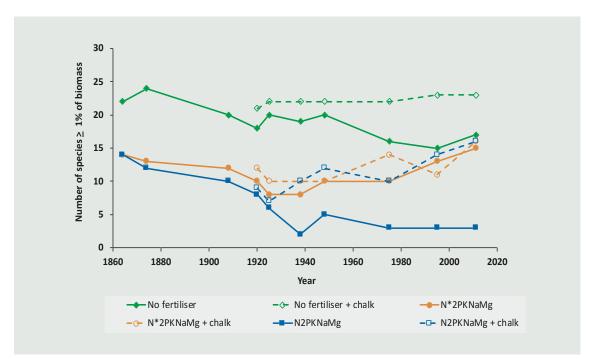
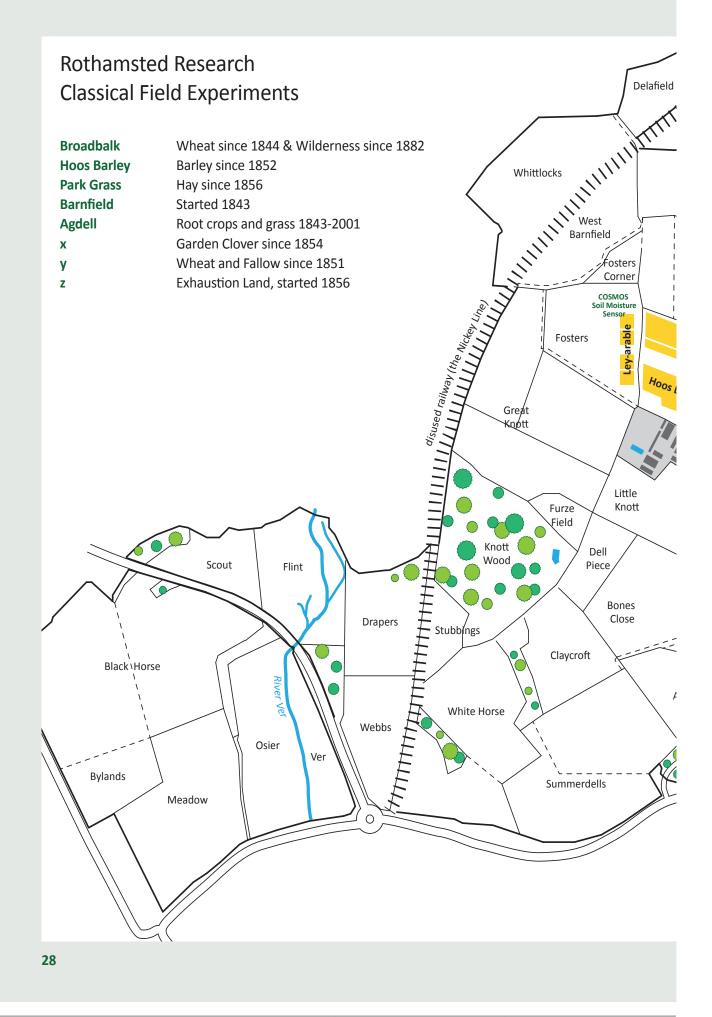
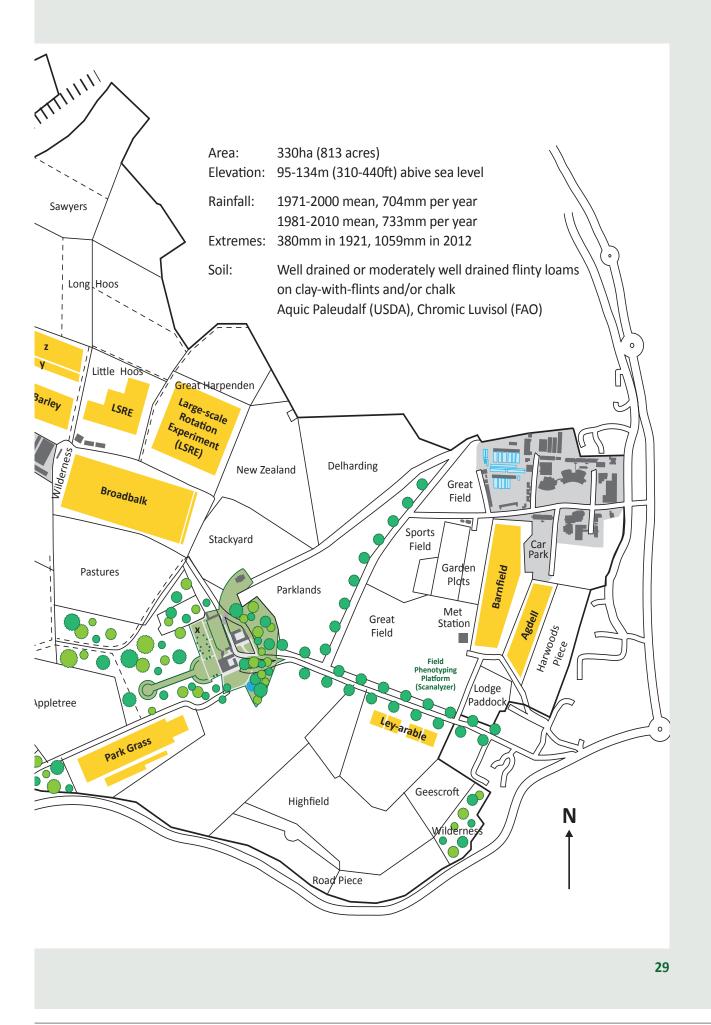


Fig. 6 Park Grass; changes in the number of species comprising 1% or more of the above-ground biomass over time, 1864-2011.



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and N fertiliser inputs. This provided the first evidence of the impact of anthropogenic stress on biodiversity in an agricultural system followed by recovery after removal of that stress (Storkey *et al.*, 2015).

Applying P alone (plot 4/1) and PNaMg (plot 8) has decreased the total number of species a little but no more than any other treatment when soils are maintained at pH 5 and above. P applications had relatively minor effects on species composition, compared to the Nil plots (data not shown), but giving K with P (plot 7), has increased the amount of dry matter from legumes, especially red and white clover (*Trifolium pratense* and *Trifolium repens*) and meadow vetchling (*Lathyrus pratensis*), thus greatly increasing yield.

The microbiology of Park Grass

The international TerraGenome consortium (Vogel et al., 2009) produced the first soil metagenome from the Park Grass untreated control plot (3d) in 2009 to examine the microbial diversity and genetic potential of the total soil microbiota. Key aims of this work were to establish the effects of different sampling approaches (spatial, temporal, depth) on variability in the soil metagenome and the application of different DNA extraction methods. The DNA extracted revealed that 89% of the DNA that could be assigned belonged to Bacteria, 1.4% to the Archaea and 1.0 % to Eukarya. The DNA extraction method was the most important factor in establishing which groups were detected and

their relative abundance; the depth, season and spatial separation were less significant. The relatively low contribution of Eukarya to the metagenome, compared to Bacteria, was surprising because fungal activity is often reported to be an important component of grassland soil ecosystems. However, such comparisons are not straightforward as soil fungi differ from bacteria in scale and growth habits, with cytoplasm-depleted hyphae connecting the actively growing tips where cytoplasm and nuclei are located.

Following the publication of the Park Grass metagenome from the control plot, different molecular approaches have been applied to study how different treatments influence the soil microbiome. A survey of 16S rRNA amplicons in community DNA collected from across the pH gradient on Park Grass plots with different N and P fertilisation regimes and controls showed that soil pH correlated most strongly with microbial diversity (H') and that the soil C/N ratio and concentration of ammonia-N also played a significant role (Zhalnina et al., 2015). A study using a nested sampling strategy on plots with and without mineral fertilisation (NPK) showed that the long-term treatments had decreased both plant and microbial α diversity (the number of different species detected) when compared to the control treatment, indicating that long-term fertilisation may magnify existing divergent spatial patterns of both plants and microorganisms.