Sea (Fig. 1, Table 1). A simple box model calculation suggests that the decreased surface water DIP from 1989 to 1998 at HOT can be caused both by decreased mixing rate between the surface and upper thermocline and by increased N_2 fixation rate. The observed decreasing SRP with increasing stratification in the Pacific is also consistent with the hypothesis that the accumulation of eolian Fe deposition in the shallow mixed layer enhances N_2 fixation, which then draws down surface water SRP.

14. Alternatively, the relative phosphate depletion in the Sargasso Sea may also be due to dominant species of phototrophs and community structure that are fundamentally different from those in the North Pacific. Although *Procholorococcus* is the dominant picoplankton genus at station ALOHA in the North Pacific, *Synechococcus* is more abundant in the North Atlantic near Bermuda. But one may argue that this phytoplankton species difference is largely due to the fact that Bermuda is located at a higher latitude than Hawaii and that community structure is not fundamentally different between the two oligotrophic gyres. The hypothesis that community structure controls DIP levels in the ocean will remain untested until information on the threshold P concentrations needed to support growth of both picoplankton genera is available.
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28. If nutrients with an N:P ratio of 25 are supplied to the surface water (DNN = 3.8 μM and DIP = 0.15 μM) and 80% of the upwelled DNN is converted to unavailable DON and the DIP is converted to refractory DOP, the net ratio of available N to P in the upward flux would be 6, well below the N:P ratio in plankton.
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30. As dissolved organic matter in the surface mixed layer has a high N:P ratio, when these dissolved organic materials mix downward and decompose in the upper thermocline, the N:P ratio in thermocline water increases. This process can raise upper ocean DIN:DIP ratios to over 16, even if organic matter produced by N_2-fixing *Trichodesmium* and non–N_2-fixing algae has an N:P ratio of 16.
31. If an N:P ratio of 16 is assumed for organic matter produced in the euphotic zone, a 50% decrease in oceanic P inventory without a concurrent decrease in N inventory would result in an N:P ratio of 32:1 in the deep ocean, and the growth of both N_2-fixing and non–N_2-fixing phototrophs will be limited by available P. The initial decreased N_2 fixation would cause an imbalance between N_2 fixation and denitrification, which would then decrease oceanic N inventory.
32. When an insufficient Fe supply decreases N_2 fixation in the presence of ample P, the rate of N_2 fixation would decline to below that of denitrification, and the ocean would start to lose fixed N. The decreased oceanic N inventory causes N limitation to primary production, which in turn would decrease water column denitrification by increasing O_2 content in the deep water until denitrification balances N_2 fixation at a new steady state. In this situation, oceanic N inventory would be set by Fe supply, not by P.
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The Response of Two Contrasting Limestone Grasslands to Simulated Climate Change

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Two different UK limestone grasslands were exposed to simulated climate change with the use of nonintrusive techniques to manipulate local climate over 5 years. Resistance to climate change, defined as the ability of a community to maintain its composition and biomass in response to environmental stress, could be explained by reference to the functional composition and successional status of the grasslands. The more fertile, early-successional grassland was much more responsive to climate change. Resistance could not be explained by the particular climates experienced by the two grasslands. Productive, disturbed landscapes created by modern human activity may prove more vulnerable to climate change than older, traditional landscapes.

The impact of climate change on the structure, composition, and function of grassland ecosystems is a topic of current concern. Climate-driven changes in grassland productivity could have serious consequences for the distribution and profitability of pastoral agriculture (*1*, *2*). Climate change will also affect the conservation value of limestone grasslands, which are among the most species-rich plant communities in Europe (*3*, *4*). Different plant communities, when exposed to changes in temperature and precipitation,

will respond in different ways and, crucially, at different rates. We define the ability of a community to maintain its composition and biomass in the face of climate change as resistance and its rate of recovery as resilience (*5*). Here, we report the resistance to simulated climate change of two limestone grasslands of strongly contrasted composition, fertility, and successional age. To simulate climate change, we applied field manipulations of local climate (*6*) to limestone grasslands at Buxton, Derbyshire, UK and Wytham, Oxfordshire, UK (Table 1).

Several properties of a plant community might influence its resistance to climate change. (i) Previous exposure to climatic extremes (*7*, *8*). For example, plant communities that frequently experience dry conditions may be more resistant to the effects of extreme droughts. (ii) Species richness. Some experimental evidence suggests that diverse plant communities are more resistant and resilient (*9*). (iii) Functional composition. Other experimental evidence demonstrates that plant traits, such as life history and growth rate, strongly influence community response to climate change and other stresses (*10*–*13*). (iv) Successional status. Mature plant communities are widely assumed to be more resistant to change than early-successional ones (*14*). These hypotheses are not mutually exclusive.

In the context of our two limestone grasslands, these hypotheses make differing predictions. It is reasonable to assume that the Wytham flora is more accustomed to, and therefore arguably more tolerant of, dry and warm conditions (Table 1) (*3*). Both sites are species-rich (Table 1); Wytham appears to contain slightly more species, but slightly fewer were detected on any single sampling occasion, probably owing to the much more temporally dynamic vegetation at that site. Therefore, in this case, species richness provides no basis for predicting response to climate change. In contrast, the floras of the two grasslands are functionally very different (Table 1). Nearly half the species at Wytham are monocarps, whereas slow-growing sedges, which make a large contribution to the biomass at Buxton, are completely absent. These differences are conveniently summarized by the mean S score, measured according to a published protocol (*15*). S scores range from −2 to +2 and measure proximity to the stress-tolerant corner of the CSR strategic triangle (*16*). Buxton is ancient, stable sheep pasture, whereas Wytham was under arable cultivation until relatively recently and is still in a dynamic, early-successional state. We might, therefore, expect Wytham to prove less resistant to climate change. Functional and successional differences between the two sites are large and suggest that Wytham should be more severely affected than Buxton. Finally, the species richness of the two sites is similar and provides no grounds for any prediction.

Vegetation dynamics at both sites were strongly influenced by a natural extreme event. The summer of 1995 was exceptionally hot and dry (*17*), although on account of the timing of recording, the full impact of this extreme weather on the plant communities was not detected until 1996. Because Wytham is in an early-successional state, all Wytham treatments (including the controls) changed significantly in composition over the five years of investigation. As far as possible, we separated these effects from those of the treatments.

We consider two aspects of resistance to climate change: biomass and species composition. Point quadrat touches are an estimate of cover and are correlated with biomass (*18*). Point quadrat data were analysed by repeated measures analysis of variance (ANOVA), with two levels of temperature (ambient and elevated), three levels of rainfall (drought, ambient, and supplemented rainfall), and five replicates (blocks). Analyses were conducted on total point quadrat touches and on various subsets (e.g., perennial grasses). Data were transformed where necessary to meet the requirements of ANOVA. At both sites, biomass in control plots fell to its lowest level in 1996 and subsequently recovered (*19*), and this interannual effect was larger than any of those due to the treatments. Relative to the plots receiving extra summer rain, control biomass fell in 1996 by 40% at Wytham and by 20% at Buxton; the corresponding falls in droughted plots were 49 and 34%, respectively. To remove the effect of this between-year variation, we plotted total biomass for each treatment as a percentage of control biomass (Fig. 1). This shows clearly that responses to climate manipulations were much more pronounced at Wytham. Also, although treatment effects were apparent at Buxton in 1996, when biomass of both droughted treatments fell relative to the control but that of watered plots did not, biomass of all treatments subsequently converged. For perennial grasses and perennial forbs at Buxton, neither of the climate manipulations (temperature or rainfall) had significant effects. Biomass of sedges, in contrast, was depressed by winter warming and substantially increased by watering in summer [$F(1, 4) = 15.98$ and $P = 0.016$; $F(2, 8) = 8.91$ and $P = 0.001$, respectively].

In contrast to Buxton, substantial treatment effects were observed at Wytham; watered and droughted plots consistently diverged in biomass (Fig. 1). Temperature and rainfall manipulations significantly affected biomass (Table 2). Significant main effects of temperature and rainfall were detected in both perennial grasses [$F(1, 4) = 11.74$ and $P < 0.05$; $F(2, 8) = 10.71$ and $P < 0.01$, respectively] and perennial forbs [$F(1, 4) = 20.33$ and $P < 0.05$; $F(2, 8) = 6.29$ and $P < 0.05$, respectively].

To examine changes in species composition, we conducted a principal components analysis (PCA) on the combined floristic data from both sites. Because the two communi-

Table 1. Comparison of characteristics of the experimental sites at Buxton and Wytham.

Characteristic	Buxton	Wytham
Origin	Ancient sheep pasture	Arable until 1982
Latitude	53° 20′	51° 46′
Altitude (m asl)	370	150
Aspect	Northwest	–
Slope	35°	level
Annual precipitation (mm)	1300	680
Mean annual temp (°C)	8	10
Extractable P (mg P/kg soil)	3.6	8.0
Total number of species recorded	60	67
Mean midsummer no. species (± SD)	29.2 (2.6)	26.1 (3.7)
Perennial grasses (no. of spp.)	16	9
Perennial dicots (no. of spp.)	46	31
Sedges (no. of spp.)	5	0
Biennials (no. of spp.)	3	5
Annuals (no. of spp.)	0	22
Mean S score (*16*)	0.83 ($n = 52$)	−0.8 ($n = 59$)
Shannon-Weiner index controls, 1994	2.4	2.0
Shannon-Weiner index controls, 1998	2.7	2.0
Shannon-Weiner index controls, all data	2.7	3.0

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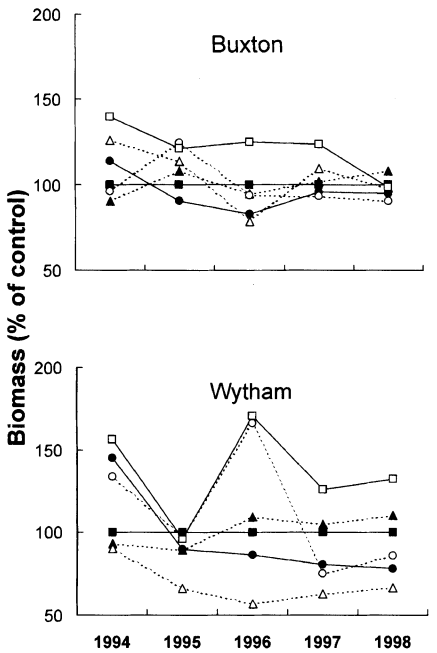

Fig. 1. Mean biomass of all treatments from 1994 to 1998, expressed as percent of control biomass. Descriptions of treatments are given in text. ■, control (C); ▲, winter warming (WW); ●, summer drought (SD); □, supplemented summer rainfall (SR), and their combinations (△, WWSD; ○, WWSR). Means (± 1 SEM) of absolute data for the main effects of the treatments are as follow (i) Buxton: C, 181.6 (12.75); WW, 183.52 (15.56); SD, 175.4 (18.78); SR, 220.36 (15.58); WWSD, 192.88 (21.52); and WWSR, 182.56 (11.55). (ii) Wytham: C, 212.52 (20.71); WW, 215.84 (25.38); SD, 199.64 (22.98); SR, 283.2 (25.43); WWSD, 145.56 (16.77); and WWSR, 226.0 (17.75).

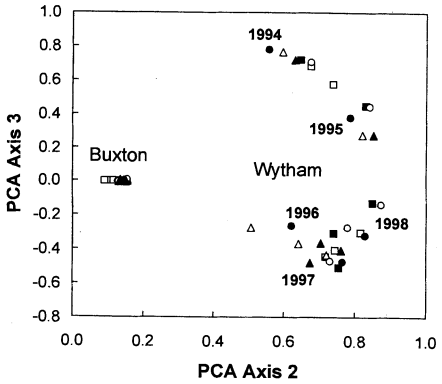

Fig. 2. PCA of the combined floristic data from Buxton and Wytham for all treatments. Proportion of variance accounted for by the PCA axes: Axis 1 (not shown), 46%; axis 2, 27%; and axis 3, 12%. Treatment symbols are as in Fig. 1. Because PCA assumes that species vary monotonically along the axes identified (*24*) and some of our data may have violated this assumption, we also conducted a detrended correspondence analysis (DCA). The results of the DCA (not shown) were qualitatively identical to those of the PCA.

ties have almost no species in common, the first axis of this PCA conveniently separated the two sites and allowed direct comparison of treatment trajectories at the two sites using PCA axes 2 and 3. The results (Fig. 2) expose clearly the very contrasted responses of the plant communities at the two sites. Over 5 years, the composition of all treatments at Buxton varied little, apart from a steady decline in abundance of sedges in response to both warming and drought. At Wytham, PCA axis 3 appears to represent a successional path being followed by all treatments. Axis 2 seems to represent treatment effects superimposed on this successional trend. A striking feature of the results is the major divergence of the watered and droughted treatments in 1996, the year of lowest biomass (Fig. 2).

Our results, therefore, support two closely related hypotheses outlined earlier. As expected from both its strategic composition and early-successional state, the plant community at Wytham was much more dynamic; it varied more over time (irrespective of treatments) and was more responsive to both the natural 1995 drought and to the experimental drought and warming treatments. There is no evidence to support the idea that resistance to climate change is conditioned by past climatic experience. The biomass of both communities was depressed by drought, especially when combined with winter heating, but this effect was larger at the warm, dry site.

Our results have implications for the future of temperate anthropogenic grasslands. Fertile or early-successional grasslands, composed of fast-growing or short-lived species, will respond rapidly to climate warming and drying; perennial grasses seem particularly at risk. More mature and/or less fertile grasslands will respond more slowly. Because expanding human populations and associated land-use changes are steadily replacing unproductive ecosystems with more fertile, disturbed ecosystems, human impacts may be making whole landscapes more responsive to climate change.

We are cautious in extrapolating from these results. Experimental warming of a subalpine meadow in Colorado, a plant community successionally and functionally similar to that at Buxton, led to a dramatic increase in shrubs at the expense of herbaceous plants (*20*). The Colorado meadow, however, was in a zone of climatic tension between these two growth forms, and the boundary between them is known to have moved in response to climate change in the past (*20*). At our sites, any major shift in growth form was prevented by management. We also emphasize that we have reported only resistance to climate change; the Buxton community might have rather low resilience, especially because most of its key species are absent from the soil seed bank (*21*).

Table 2. ANOVA results for total biomass at Wytham, showing significant main effects of both the temperature and rainfall manipulations. Such effects did occur for the perennial grasses and perennial dicots at Wytham and the sedges at Buxton. Main effects of treatments were not significant for total biomass at Buxton and for all other groups at both sites. Significant main effects from the repeated measures ANOVA were interpreted as consistent long-term impacts of the treatments. Treatment × date interactions, which may be open to a variety of interpretations, are not discussed here. 1, winter warming; 2, summer rainfall manipulations; 3, block; 4, date. Only significant *P* values (<0.05) are shown.

Effect	df (effect)	MS	df (error)	MS (error)	*F* value	*P* value
1	1	52416.1	4	728.12	71.99	0.001
2	2	131645.5	8	14154.52	9.3	0.008
3	4	35671.1	–	–	–	
4	4	80605.1	16	7190.08	11.21	<0.001
1 × 2	2	15705.3	8	19392.45	0.81	
1 × 4	4	1818.9	16	2833.97	0.64	
2 × 4	8	12933.5	32	2665.55	4.85	<0.001
1 × 2 × 4	8	4287.4	32	2501.16	1.71	
1 × 2 × 3 × 4	32	2501.2	–	–	–	

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which were spare, and the remainder containing one each of elevated winter temperature, controlled summer drought, supplemented summer rainfall, elevated winter temperature and summer drought, elevated winter temperature and supplemented summer rainfall, control, and cable control (ambient conditions with unconnected heating cables). At the end of the growing season, all plots were cut to a height of 4 to 5 cm to maintain a short turf. At Buxton, point quadrat surveys were conducted on four occasions per year, with the first in April and the last in late September, from 1994 to 1997. During the same period, vegetation surveys took place every 6 weeks at Wytham. More recently (1998 onwards) point quadrat sampling has been conducted only once (Buxton) or twice (Wytham) per year. Analysis was restricted here to data collected each year in late June/early July, the time of maximum plant growth and immediately before the imposition of the drought treatment. Thus, the data collected in any year do not reflect the immediate effects of the drought treatment applied in that year.

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REPORTS

CikA, a Bacteriophytochrome That Resets the Cyanobacterial Circadian Clock

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The circadian oscillator of the cyanobacterium *Synechococcus elongatus*, like those in eukaryotes, is entrained by environmental cues. Inactivation of the gene *cikA* (circadian input kinase) shortens the circadian period of gene expression rhythms in *S. elongatus* by approximately 2 hours, changes the phasing of a subset of rhythms, and nearly abolishes resetting of phase by a pulse of darkness. The CikA protein sequence reveals that it is a divergent bacteriophytochrome with characteristic histidine protein kinase motifs and a cryptic response regulator motif. CikA is likely a key component of a pathway that provides environmental input to the circadian oscillator in *S. elongatus*.

The cyanobacterium *S. elongatus* PCC 7942 (*1*) exhibits circadian rhythms of gene expression that can be monitored using luciferase reporter genes (*2*). These bioluminescence rhythms persist with a period of approximately 24 hours, are temperature compensated, and their phase can be reset by light/dark transitions or by temperature cues (*3*). The cyanobacterial clock exhibits these characteristics of eukaryotic circadian clocks despite a lack of apparent homology between its protein components and those identified in other groups of organisms (*4*). For example, the complete genome sequence of *Synechocystis* sp. strain PCC 6803 is devoid of sequences similar to clock genes of *Drosophila*, such as *period*, *timeless*, *Clock*, and *cycle*, or the *frequency* gene of *Neurospora* (*4*, *5*). Likewise, no homologs of the cyanobacterial *kaiA*, *kaiB*, or *kaiC* genes, essential for circadian rhythmicity (*6*), have been detected thus far in eukaryotes. Other cyanobacterial genes that, when mutated, affect relay of temporal information from the clock to downstream genes include a sigma factor (*7*) and a putative carboxylase (*8*). A histidine protein kinase, SasA, interacts with the KaiC protein and works with the oscillator either at a point of environmental input or of output transduction to all downstream genes (*9*). We describe here a new clock-associated gene, *cikA*, that lies on an input pathway that supplies phase-setting information to the *S. elongatus* clock.

The *cikA* gene was identified from a Tn5 transposon insertion mutant (*2*) that showed subtle alteration in light-responsive regulation of a photosystem II gene, *psbAII* (*10*). Expression of a *psbAII*::*luxAB* (bacterial luciferase) fusion in the mutant was 50 to 80% of wild type under low light conditions and showed exaggerated induction on exposure to higher light intensity (*11*). However, a more striking circadian (*2*, *12*) phenotype was noted: the period of bioluminescence oscillation was shortened by approximately 2 hours (22.80 ± 0.45 versus 24.71 ± 0.25, $n = 12$), and the relative timing of peaks (phase angle) was offset by approximately 6 hours (Fig. 1A).

Reduction of both period and amplitude was observed with all reporters (Fig. 1, A to D) (e.g., periods for *kaiB*::*luxAB*, 22.36 ± 0.47 hours versus 25.24 ± 0.35 hours, $n = 12$; for *purF*::*luxAB*, 22.75 ± 0.24 hours versus 24.86 ± 0.33 hours, $n = 12$). Nonetheless, expression from the *kaiB* promoter, indicative of clock gene expression, remained robustly rhythmic with no notable alteration in phase angle (Fig. 1B). The bioluminescence rhythm from a *purF*::*luc* reporter (firefly luciferase) was also affected in both amplitude and period (Fig. 1C), indicating that the phenotype is not related to the substrates of bacterial luciferase and that it extends to class 2 genes [*purF* peaks at subjective dawn and is defined as class 2; the majority of gene expression patterns in the organism peak near subjective dusk and are defined as class 1 (*13*)]. A gentamycin resistance cassette inserted in both orientations with respect to the *cikA* open reading frame (ORF) caused phenotypes identical to those of the original Tn5 insertion mutant (Fig. 1D). Note that the *kaiA*::*luxAB* reporter showed an altered phase-angle phenotype; thus, in the *cikA* genetic background, the relative phasing of *kaiA* and *kaiBC* expression is uncoupled without dramatically affecting circadian timing (Fig. 1, B and D), as was previously demonstrated for mutation of the *cpmA* gene (*8*).

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