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Mycorrhizal fungal abundance is affected by long-term climatic manipulations in the field

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Abstract

Climate change treatments – winter warming, summer drought and increased summer precipitation – have been imposed on an upland grassland continuously for 7 years. The vegetation was surveyed yearly. In the seventh year, soil samples were collected on four occasions through the growing season in order to assess mycorrhizal fungal abundance. Mycorrhizal fungal colonisation of roots and extraradical mycorrhizal hyphal (EMH) density in the soil were both affected by the climatic manipulations, especially by summer drought. Both winter warming and summer drought increased the proportion of root length colonised (RLC) and decreased the density of external mycorrhizal hyphal. Much of the response of mycorrhizal fungi to climate change could be attributed to climate-induced changes in the vegetation, especially plant species relative abundance. However, it is possible that some of the mycorrhizal response to the climatic manipulations was direct – for example, the response of the EMH density to the drought treatment. Future work should address the likely change in mycorrhizal functioning under warmer and drier conditions.

Keywords: arbuscular mycorrhizas, climate change, drought, global environmental change, warming

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Introduction

Human activity now has discernible effects on the Earth's climate (Kerr, 2001). These effects will continue through the next decades and will result in a substantially warmer planet with altered weather patterns (Houghton *et al*., 1995). Many global circulation models predict that temperate areas will become warmer and drier (Cao & Woodward, 1998), with more extreme events such as severe droughts (Easterling *et al*., 2000). There is a considerable amount of research, which aims at understanding the effects of human-induced environmental change, and climate change in particular, on the Earth's ecosystems (e.g. Oechel *et al*., 2000). However, most of this research is focused on the aboveground component of the vegetation with rather little effort being targeted at belowground aspects. The soil ecosystem is an integral part of terrestrial ecosystems (Coleman & Crossley, 1996). Soil, including its biota, has numerous ecosystem functions (Killham, 1994) – for example, nutrient cycling – and plays a crucial role in the terrestrial and global carbon cycles (Schimel, 1995). Soil is, therefore, involved in feedback mechanisms to global environmental change (Davidson *et al*., 2000; but see also Luo *et al*., 2001), which is driven primarily by anthropogenic greenhouse gases, including carbon dioxide (CO_2), released from the burning of fossil fuels (Wyman, 1991).

How soil organisms will respond to climate change is not known. In fact, very little is known about soil biodiversity and function per se (Killham, 1994). Of the many various types of soil organisms, mycorrhizal fungi could be considered as a key group. Mycorrhizal fungi form symbiotic associations with plant roots: the fungus receives its carbon from the plant and provides the plant with nutrients, especially phosphorus (P), and other

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benefits such as protection from pathogens (Newsham *et al*., 1995). Arbuscular mycorrhizas are the most common type of mycorrhizas and are found in most plant species (Smith & Read, 1997). They are particularly common in temperate grasslands. Mycorrhizal fungi mediate competition between plant species (Hetrick, 1991) and influence plant community structure (van der Heijden *et al*., 1998). Perhaps, more importantly in terms of the global carbon cycle, mycorrhizas can account for a large proportion of the carbon fixed by the plants: up to 20% in some cases (Jakobsen & Rosendahl, 1990). Also, the extensive mycorrhizal hyphal network in the soil is likely to provide an important pathway for the flow of carbon from roots to bulk soil (Staddon, 1998). There has been surprisingly little research undertaken concerning the effects of climate change on arbuscular mycorrhizas (Fitter *et al*., 2000). Virtually nothing is known on how mycorrhizal fungi might respond to long-term environmental change in the field. The response of mycorrhizal fungi to environmental changes such as climate changes must be better understood if we are to predict with greater certainty how terrestrial ecosystems will respond to future environmental changes.

The main objective of the work presented here is to determine whether predicted climate change, specifically increased winter temperature and altered summer precipitation, alters mycorrhizal abundance in the field, and to what extent these effects of climate change on mycorrhizas are simply mediated by changes in the vegetation. This research was based at the Buxton Climate Change Impacts Laboratory (BCCIL), Derbyshire, UK (Grime *et al*., 2000; www.shef.ac.uk/~nuocpe/bccil/), where a long-term climatic manipulation experiment is now in its seventh year. We briefly assess the effects of climatic manipulation on the vegetation and attempt to test the following specific hypotheses:

- Climate change – both winter warming and altered summer precipitation – alters mycorrhizal fungal abundance.
- The effect of climate treatment on mycorrhizal fungal abundance is mediated, at least in part, by a change in the vegetation.

Materials and methods

Site, climate treatments and vegetation

The BCCIL site and climate treatments are fully described in Grime *et al*. (2000). The site is an ancient upland limestone sheep pasture at Buxton, Derbyshire, UK. The climate treatments were started in November 1993 and were at the time of the work reported in this paper in their seventh year.

From November to April inclusive, winter temperature was elevated by 3 °C above ambient by heating cables (Camplex Thermoforce Ltd) fastened to the soil surface. The heating intensity was controlled by a computer system linked to temperature probes. During July and August, summer drought was imposed by excluding all rainfall with automatically operated rainshelters, which slide across the plot when it rains. From June to September inclusive, summer water addition was imposed at regular intervals in order to reach the equivalent of 20% increase in rainfall compared to the previous 10-year average; refer to Grime *et al*. (2000) for further details. A fully randomised block design, each containing nine $3 \times 3\,m^2$ plots, was used, replicated five times. Each block contained the following treatments: (i) control, (ii) summer drought, (iii) summer water addition, (iv) winter warming (or heating), (v) winter warming and summer drought, (vi) winter warming and summer water addition, (vii) cable control (unconnected warming cables) and two spare plots. The vegetation in the plots was cut to a height of 4–5 cm at the end of each growing season.

Vegetation surveys were performed at regular intervals (see Grime *et al*., 2000), the latest point quadrat sampling occurred in June 1999 and June 2000, immediately prior to the start of the yearly drought treatment. Total quadrat point count was used as an estimate of standing plant biomass. Two indices of plant diversity were used:

- Simpson $D = 1/\Sigma P_i{}^2$ (richness, dominance) and
- Shannon–Wiener $H = -\Sigma P_i \,.\, \ln P_i$ (evenness),

where P_i is the proportion of individual counts for species 'i' (calculations follow Begon *et al*., 1990).

Collection of belowground data

Soil cores were collected on four occasions over the growing season from the six main treatments (the cable control treatment was excluded from the sampling as the previous unpublished data had shown that there was no detectable difference between the cable control and the control treatments). Harvests occurred on 3 May 2000, immediately after the end of the winter warming treatment; on 30 June 2000, prior to the onset of summer drought; on 20 September 2000, immediately after the end of summer drought; on 14 November 2000, at the start of winter warming. Duplicate cores – 2 cm in diameter and up to 10 cm deep (when possible) – were collected from each of the treatments in each of the blocks (in total 60 cores per harvest). Cores were sealed in plastic bags and refrigerated at 5 °C until processed, which varied from 1 to 14 days for individual cores. The storage

of the cores up to 2 weeks was not a problem as we were not assessing the vitality of mycorrhizas but rather 'total' mycorrhizal parameters, which we have previously shown to remain relatively constant under such conditions (see Staddon & Fitter, 2001).

Each core was processed for measurements of extraradical mycorrhizal hyphal length (EMH) density, percent root length colonised (RLC) and root weight density as follows. A subsample of soil was fresh-weighed and then placed in 500 mL of water. This was then mixed using a magnetic stirrer, and diluted accordingly depending on the concentration of the soil solution. From the final solution, duplicate 5 mL samples were taken and passed through 0.45 μm filters under vacuum for extraradical mycorrhizal hyphae (EMH) collection (for a full description of the procedure, see Staddon *et al*., 1999). Roots were extracted from the soil and dried at 75 °C for a minimum of 3 days. A small random subsample of roots was separated from the main bulk of the roots prior to drying and stained with acid fuchsin for internal mycorrhizal assessment. The timing of the staining procedure was 2–3 min in potassium hydroxide (KOH) at 80 °C, 1 min in hydrochloric acid (HCl) at room temperature, 25 min in acid fuchsin at 80 °C, followed by destaining in lactoglycerol (for a full description of the procedure, see Staddon *et al*., 1998). Both the filters containing the EMH and the stained roots were mounted in lactoglycerol onto microscope slides.

In order to allow for conversion of soil fresh weight (FW) to soil dry weight (DW), the remaining soil from each core was fresh-weighed and then oven-dried at 75 °C. This involved all the cores from harvests 3 and 4, but only a random subset from harvests 1 and 2. Soil moisture (percent water content) data is, therefore, available for harvests 3 and 4, immediately after the end of the summer drought and 2 months after.

Extraradical mycorrhizal hyphal density was assessed using a compound microscope (Zeiss Jenamed 2) fitted with a $1 \times 1\,cm^2$ 100-grid graticule (Graticules Ltd, UK) at $\times 250$ with a minimum of 30 grids per filter being observed. The grid-line intercept method (see Tennant, 1975) was used in order to obtain a length of hyphae per filter. The mean value of the four filters (two filters per duplicate) per replicate was used. Hyphal density is finally expressed in m hyphae per g soil DW. Hyphae were counted as mycorrhizal if they showed the following typical characteristics: dichotomous branching, angular projections, absence of septa (Nicolson, 1959). This standard method (e.g. Miller *et al*., 1995; Kabir *et al*., 1997; Schweiger & Jakobsen, 1999; Rillig *et al*., 2002) may slightly underestimate EMH density by excluding some hyphae, which may actually be mycorrhizal. Internal mycorrhizal colonisation was assessed using a compound microscope (Nikon EFD-3 Optiphot-2) fitted with a cross-hair graticule at $\times 200$ with epifluorescence (Merryweather & Fitter, 1991); a minimum of 80 intersections were assessed per core. Scoring followed McGonigle *et al*. (1990). The mean value for the two cores per replicate was used. Further details on the methods used for collection of mycorrhizal data are available in Staddon *et al*. (1998, 1999).

Statistical analysis

All statistical analyses were performed using SPSS 10.0. All data was checked for normality and where necessary was ln transformed (EMH density, root density, EMH/root ratio) or square root arcsine transformed (RLC, percent species cover). Correlation analysis was used in order to test for relationships between variables (EMH, RLC, root density, plant diversity, plant species cover, soil moisture, etc.). Repeated measures analysis of variance (ANOVA) and repeated measures analysis of covariance (ANCOVA) were used in order to test for climate treatment effects on the various measured variables, including plant diversity.

Results

Effects on the vegetation

Climate treatments had no effect on plant biomass. However, plant diversity (both Simpson *D* and Shannon–Wiener *H* indices) was significantly decreased by both winter warming and summer drought ($P < 0.001$); summer water addition had little effect (Fig. 1). For example, winter warming decreased the June 2000 Simpson *D* index from 8.1 (nonheated plots) to 5.3 (heated plots); similarly, summer drought decreased the June 2000 Simpson *D* index from 7.8 (plots with no water treatment) to 4.6 (droughted plots). Both winter warming and summer drought increased *Festuca* cover ($P < 0.001$) and

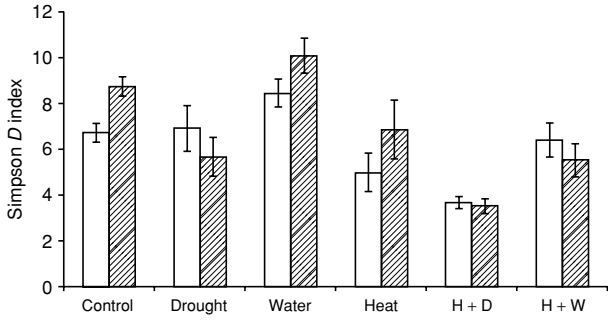

Fig. 1 Plant diversity index as affected by climatic manipulations. Simpson *D* index of diversity data is presented, but a similar pattern is seen with the Shannon–Wiener *H* index. Empty bars: June 1999; hashed bars: June 2000; error bars represent standard errors.

decreased *Carex* cover ($P < 0.05$), although the effects were much stronger for *Festuca* than for *Carex*.

Festuca ovina and *Carex* species – *C. flacca*, *C. caryophyllea*, *C. panicea* and *C. pulicaris* in declining order of abundance – were the dominant species at the site, with *Festuca* cover ranging from 19% in the summer water addition (W) treatment to 51% in the winter warming and summer drought (HD) treatment and *Carex* cover ranging from 12 to 32% in the HD and W treatments, respectively. *Festuca* and *Carex* cover were negatively correlated ($P < 0.001$). *Festuca* cover was negatively correlated with plant diversity ($P < 0.001$) and positively correlated with plant biomass ($P = 0.029$), whereas *Carex* cover was positively correlated with plant diversity ($P = 0.001$) and negatively correlated (although only a trend) with plant biomass ($P = 0.066$).

Two measures of soil moisture are available: 20 September (H3) and 14 November 2000 (H4). Note that the H3 measure taken in September immediately after the end of the summer drought treatment was strongly linked to the water regime, whereas this was not the case for the H4 (November) measure (Fig. 2). Plant diversity was positively correlated with H3 soil moisture ($P < 0.01$) but not significantly so with H4 soil moisture. In contrast, plant biomass was negatively correlated with H4 soil moisture ($P < 0.01$) but not with H3 soil moisture. *Festuca* cover was negatively correlated with soil moisture ($P = 0.004$ at H3 and $P = 0.061$ at H4), whereas *Carex* cover was not (positive trend only). In other words, it is not that *Carex* species were strongly associated with soil moisture (as might be expected), but rather that *Festuca ovina* was strongly negatively correlated with soil moisture.

In June 1999, there was no correlation between plant biomass and plant diversity; however, in June 2000 there was a significant negative correlation between plant biomass and the Shannon–Wiener *H* diversity index ($P = 0.050$; $P = 0.068$ for Simpson *D*). Plant diversity was strongly correlated between the two years ($P < 0.001$); however, there was surprisingly no relationship between the estimate of plant biomass for the two years.

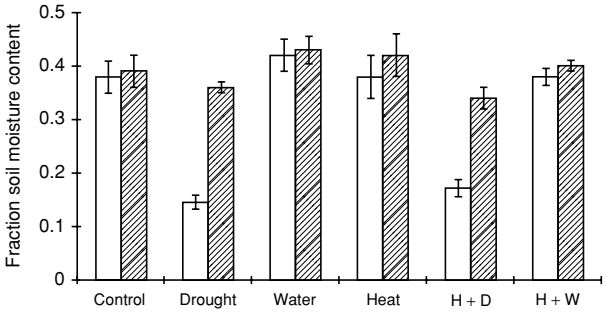

Fig. 2 Soil moisture content (SMC) as affected by climatic manipulations. Empty bars: 20 September 2000; hashed bars: 14 November 2000; the September measure was immediately after the end of the summer drought treatment; error bars represent standard errors.

Belowground effects: mycorrhizas and roots

There were significant effects of the climate treatments on both mycorrhizas and roots (Table 1). Percent root length colonised RLC was increased by both summer drought ($P < 0.001$) and winter warming ($P < 0.05$) (Fig. 3). However, EMH length density was decreased by both summer drought ($P < 0.001$) and winter warming ($P < 0.01$) (Fig. 4). Similarly, root weight density was decreased by both summer drought ($P < 0.05$) and winter

Table 1 The level of significance for the between-subjects effects of water and temperature treatments on mycorrhizal and root parameters as obtained by repeated measures analysis of variance (ANOVA). The water factor has three levels: control, water addition and drought; the temperature factor has two levels: control and heating. Post hoc tests showed that the drought treatment was responsible for most of the water factor's significant effects – that is, the control and water addition treatments were very similar

	Water	Temperature	Interaction
Percent root length colonised (RLC)	***	*	*
Extraradical mycorrhizal hyphal (EMH) length density	***	**	*
Root weight density	*	*	NS
EMH length to root weight ratio	NS	NS	NS

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS = not significant.

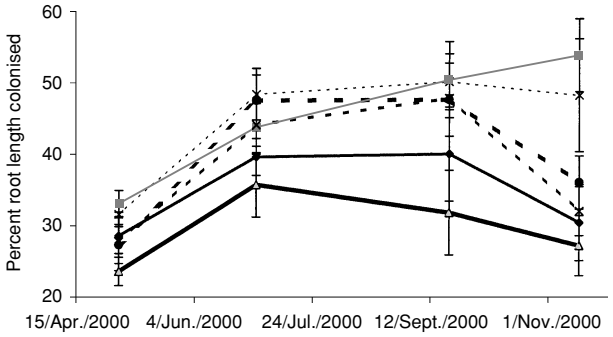

Fig. 3 Root length colonised (RLC) (percent) by arbuscular mycorrhizal fungi as affected by climatic manipulations. Treatments are: control (▬▬), summer drought (——), summer water addition (▬▬), winter warming (▬ ▬), warming and drought (- - - -), warming and water addition (▬ ▬); error bars represent standard errors.

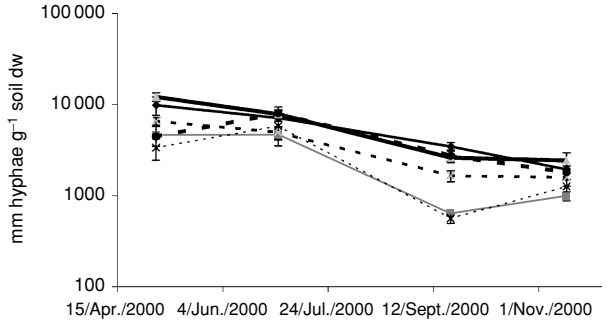

Fig. 4 Extraradical mycorrhizal hyphal (EMH) density (mm hyphae g^{-1} soil dw) as affected by climatic manipulations. Note the log 10 scale. Treatments are: control (——), summer drought (——), summer water addition (——), winter warming (– –), warming and drought (- - - -), warming and water addition (– –); error bars represent standard errors.

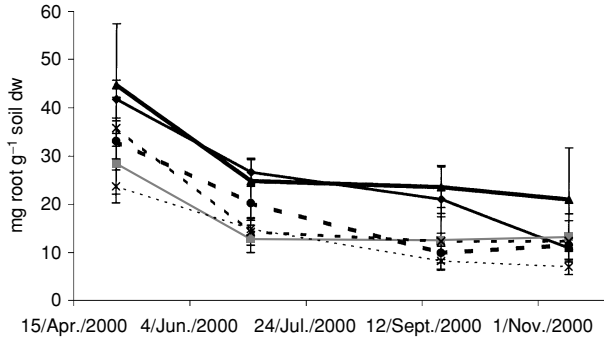

Fig. 5 Root weight density (mg root g^{-1} soil dw) as affected by climatic manipulations. Treatments are: control (——), summer drought (——), summer water addition (——), winter warming (– –), warming and drought (- - - -), warming and water addition (– –); error bars represent standard errors.

warming ($P < 0.05$) (Fig. 5). Neither treatment type (temperature or precipitation) had any obvious effect on the ratio of EMH length to root weight (Fig. 6). There was a significant interaction ($P < 0.05$) between the winter warming and summer water manipulation treatments on EMH and RLC, possibly involving differential effects of the water addition treatment depending on the winter warming treatment. Inclusion of moisture as a covariate minimally altered these findings, as did plant biomass (when covariate significant). Plant diversity was never significant as a covariate and generally nor was *Festuca* or *Carex* cover.

Extraradical mycorrhizal hyphal length and root weight density were positively and percent RLC negatively correlated with plant diversity (Table 2). However, none of the mycorrhizal and root variables were correlated with plant biomass. Extraradical mycorrhizal hyphal length and root density were generally positively correlated with *Carex* cover and negatively with *Festuca*

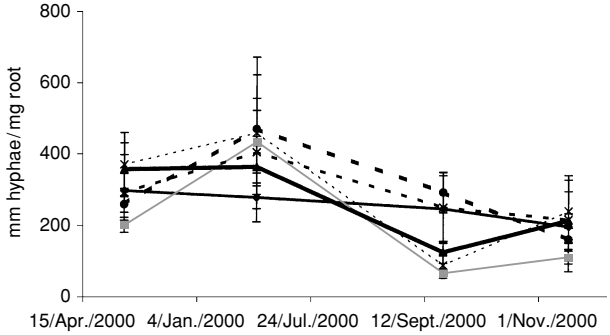

Fig. 6 Ratio of extraradical mycorrhizal hyphae (EMH) to root (mm/mg) as affected by climatic manipulations. Treatments are: control (——), summer drought (——), summer water addition (——), winter warming (– –), warming and drought (- - - -), warming and water addition (– –); error bars represent standard errors.

Table 2 Correlations between mycorrhizal and root parameters and plant diversity based on vegetation data from June 2000 (June 1999 data gave similar results). All dates refer to 2000

	Percent root length colonised (RLC)	Extraradical mycorrhizal hyphal (EMH) density	Root weight density	EMH length to root weight ratio
3 May	NS	+***	+**	+**
30 June	–***	NS	+*	NS
20 September	–*	+***	+*	NS
14 November	–*	+**	NS	NS

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS = not significant; + positive correlation; – negative correlation.

cover, but for RLC the opposite was true (Table 3). The ratio of EMH length to root weight was not significantly correlated with either *Carex* or *Festuca* cover. The only exception to this was the 3 May harvest, where there was a negative correlation between the ratio of EMH length to root weight and *Festuca* cover. Of note is that the increase in percent RLC and decrease in EMH length density by the summer drought and winter warming treatments corresponded with an increase in the cover of *Festuca* and *Koeleria macrantha* and a decrease in that of *Carex* and *Potentilla erecta*. *Festuca* and *Koeleria* cover increased from a combined cover of 23 and 21% in the control (C) and the summer water addition (W) treatments, respectively, to a maximum of 56% in the winter warming and summer drought (HD) treatment; *Carex* and *Potentilla* cover decreased from a combined maximum cover of 33 and 39% in C and W to a minimum of 13% in HD.

Extraradical mycorrhizal hyphal length density was positively correlated with root weight density ($P < 0.001$), but negatively correlated with per cent RLC ($P < 0.001$),

Table 3 Correlations between mycorrhizal and root parameters and *Carex* and *Festuca* cover based on vegetation data from June 2000. All dates refer to 2000

		Carex cover	*Festuca* cover
Percent root length colonised (RLC)	03 May	NS	NS
	30 June	$-^{*}$	$+^{***}$
	20 September	$-^{\dagger}$	$+^{*}$
	14 November	$-^{*}$	$+^{*}$
Extraradical mycorrhizal hyphal (EMH) length density	03 May	NS	$-^{***}$
	30 June	$+^{*}$	NS
	20 September	$+^{*}$	$-^{***}$
	14 November	$+^{**}$	$-^{**}$
Root weight density	03 May	$+^{\dagger}$	$-^{**}$
	30 June	NS	$-^{*}$
	20 September	NS	$-^{*}$
	14 November	$+^{*}$	$-^{\dagger}$
EMH length to root weight ratio	03 May	NS	$-^{**}$
	30 June	NS	NS
	20 September	NS	NS
	14 November	NS	NS

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{\dagger}P < 0.10$; NS = not significant; + positive correlation; – negative correlation.

which was also negatively correlated with root weight density ($P < 0.001$). Extraradical mycorrhizal hyphal and root density were both positively correlated with soil moisture content ($P < 0.001$ and $P = 0.001$, respectively), but RLC was negatively correlated with soil moisture ($P < 0.001$).

Discussion

The vegetation

As reported previously for vegetation data until 1996 (Grime *et al*., 2000), there was no effect of the various climatic manipulations, which started in November 1993, on total plant biomass in 1999 or 2000. Grime *et al*. (2000) also reported minimal changes in the vegetation until 1996. However, by 1999, there were highly significant effects of the imposed climate treatments on plant diversity as measured by both the Simpson *D* index of dominance and the Shannon–Wiener *H* index of evenness. Both winter warming and summer drought substantially decreased plant diversity. A similar phenomenon has been reported for a subalpine Colorado meadow (Harte & Shaw, 1995). However, at the Colorado site it was an increase in a shrub, *Artemisia tridentata*, which resulted in lower plant diversity under the drier and warmer conditions.

This change in plant diversity at the Buxton site was correlated with a strong increase in the percentage cover of the dominant grass *Festuca ovina* and a decrease in the cover of the sedges – principally *Carex flacca* – in both the winter warming and the summer drought treatments. Soil moisture appeared to be a determining factor for *Festuca* cover, but not for *Carex* cover. This would suggest that, in the case of the Buxton site, the soil would still be sufficiently wet after the climatic manipulations to support *Carex* over its entirety but that the winter warming and summer drought allowed *Festuca* to become more competitive. The winter warming treatment – particularly when combined with summer drought – shifted the growing season away from the summer towards the spring and autumn. Grasses seemed to be able to take advantage of this shift, whereas sedges did not. This is likely to be because sedge growth is confined to the warmest parts of the year, whereas grass growth is not (Grime *et al*., 1985). The increased competitive ability of *F. ovina* and some of the other grasses – for example, *Koeleria macrantha* – under the warmer and drier conditions was likely to be the main cause for the decrease in occurrence of many of the less common forbs such as *Lotus corniculatus* and *Plantago lanceolata*. It also resulted in the overall decrease of the number of rarer species.

The location of the site was likely to have played a major role in the measured outcomes of the climate treatments on plant diversity. The availability of a pool of species, which can take advantage of changing climatic conditions when they occur, is a crucial factor in determining how an ecosystem will respond (e.g. Buzas & Culver, 1994). In a manipulation experiment such as the one reported here, there may well be no available pool of species with the potential to invade. This, it could be argued, reflects the current environmental conditions of fragmented habitats (see Casagrandi & Gatto, 1999).

Mycorrhizas and roots

There were significant effects of the climate treatments on both the mycorrhizas and roots. The overall level of RLC by arbuscular mycorrhizal fungi at the site was stimulated by both summer drought and winter warming. This observation that drought promoted more extensive mycorrhizal colonisation has been noted in other field studies (Augé, 2001). However the opposite was true for the density of both EMH and roots. Similar effects of drought on RLC and EMH have been noted previously (unpublished data) during an experiment performed in growth chambers. Both in the field and in the laboratory, soil moisture content (SMC) was a key factor in determining the relative abundance of mycorrhizal fungi within and outside plant roots. This does not mean that SMC affects mycorrhizal fungi directly; the effect could be via a change in root density and or vegetation composition (see next section). Overall, RLC at the Buxton site was negatively correlated with EMH suggesting that there

was a trade-off on the part of the mycorrhizal fungus between investing in root occupation and soil exploration. This fits well with the theory that under stressed conditions – for example, drought – a mycorrhizal fungus will invest more resources in storage capacity in roots and less in external hyphae (Smith & Smith, 1996). However, an alternative explanation for the negative correlation between RLC and EMH could be the change in relative abundance of roots belonging to mycorrhizal versus nonmycorrhizal plant species (see next section).

The climatic manipulations interacted with each other to some degree in their effects on mycorrhizal parameters. This was particularly so for the heating and water addition treatments: water addition decreased RLC only under nonheated conditions and increased EMH density only under heated conditions. Presumably these interactions would have been because of changes in the vegetation, which would have occurred under warmer and wetter conditions (see next section).

Root weight density exhibited the same pattern of response to the climatic variables as EMH length density. This resulted in a relatively stable EMH to root ratio across all treatments. Although this ratio is of a length to a biomass (m/g) it is nonetheless informative in terms of carbon partitioning between host plants and mycorrhizal fungi (see Read, 1992). A stable ratio would indicate that, despite severe climatic manipulation, the proportion of belowground carbon invested in standing root or EMH structures was unchanged; or in other words, carbon partitioning between root and EMH was not affected by the climate treatments. A caveat to this is that root turnover may have been altered by the climatic manipulation, as has been reported previously for a different upland grassland (Fitter *et al*., 1998); if this occurred at the Buxton site then EMH turnover might also be altered. Nonetheless, this observation that the carbon partitioning between root and EMH was rather stable has been noticed previously in the context of elevated atmospheric CO_2: any change in mycorrhizal parameters was proportional to changes in plant size (Staddon & Fitter, 1998). This would also support the theory that the mycorrhizal fungi simply responded to the amount of carbon available to them and had rather little control over the amount they can obtain from the host plant (Tester *et al*., 1986).

As noted above, a caveat to this is that in this climate change experiment, the quantity of mycorrhizal fungi inside the roots was affected inversely to that outside the roots. This would indicate that the fungi were altering their within carbon allocation pattern between structures inside and outside of the roots leading to an increase in the ratio of internal to extraradical mycorrhizal carbon in the summer drought and winter warming treatments. This would also mean that, although the ratio of standing EMH to root was unaffected by the climate treatments, the amount of carbon partitioned to the fungi in the mycorrhizal associations was increased in the drought and warming treatments. A question that arises here is whether, or more likely how, the turnover of root and mycorrhizal structures was affected by the climatic manipulations. Indeed, fine roots have been shown to alter their turnover under changed climatic conditions (Fitter *et al*., 1997). As far as we are aware, nothing is known about the environmental effects on mycorrhizal turnover.

Are mycorrhizal effects dependent on vegetation changes?

Mycorrhizal parameters were correlated with plant diversity and the percent cover of the dominant grasses and sedges. Generally the direction of the correlations with vegetation parameters was opposite for RLC and EMH. The increase in RLC with increasing cover of *Festuca* and *Koeleria* and decreasing cover of *Carex* and *Potentilla* (and overall decrease in plant diversity) is not surprising when the mycorrhizal status of the plants is taken into account: both *Festuca* and *Koeleria* are mycorrhizal, *F. ovina* can be quite strongly so, whereas *Carex* species are generally nonmycorrhizal (occasionally, some species can be moderately mycorrhizal) and *Potentilla* is known to be facultatively mycorrhizal (Harley & Harley, 1987).

The negative correlation between RLC and EMH could, therefore, be explained in terms of the change in relative abundance of roots belonging to mycorrhizal versus nonmycorrhizal plant species under the climatic manipulations. Although the summer drought and winter warming treatments resulted in an increase in percent *Festuca* cover, and therefore also a likely increase in the proportion of *Festuca* roots, the overall root weight density decreased. So, under the drought and warming treatments, the greater proportion of *Festuca* roots resulted in the overall increase in the percentage of RLC; and the lower total root density (and concomitant lower absolute RLC) resulted in the decrease in EMH density. However, assuming no major change in specific root length at the site, an increase in percent root length colonisation with no change in the ratio of EMH to root would mean that the ratio of internal to external mycorrhizal structures was increased. If this is indeed what is happening, this would support the theory of increased mycorrhizal fungal carbon allocation to structures inside roots under conditions of environmental stress (Smith & Smith, 1996). This could be either as a result of changes in carbon partitioning within an 'individual' mycorrhizal fungus or as a result of possible changes in the mycorrhizal fungal community.

Another possible explanation as to why EMH density does not follow RLC is that EMH were more directly affected by the edaphic conditions, especially soil moisture (Augé, 2001: Table 6) but also soil temperature. In other words, there are two mechanisms operating: the direct effects of edaphic conditions on EMH and the indirect effects mediated by altered plant community composition. So, in the case of this experiment, summer drought – for example – favoured strongly mycorrhizal plants on the one hand but on the other hand resulted in a decline in EMH density relative to the amount of mycorrhizal colonised root (as the ratio of EMH length to root weight was unchanged and assuming no change in specific root length). Any decrease in EMH growth as a direct result of edaphic factors would have resulted in more mycorrhizal carbon being available for intraradical mycorrhizal structures. However, from our data it is not possible to distinguish whether there were truly any direct edaphic effects on the EMH. There is also the possibility that various mycorrhizal fungal species were favoured in the drought and warming treatments either as a result of altered plant species abundance or as a direct result of altered edaphic conditions.

Conclusions

Mycorrhizal fungi responded to long-term climatic manipulations in the field, including a change in both soil temperature and moisture content. The most significant mycorrhizal fungal response was to drought, where the proportion of root length colonised (RLC) was increased and the extraradical mycorrhizal hyphal (EMH) density was decreased. Much of the mycorrhizal fungal response to climate change was attributed to vegetation changes. However, changes in plant species composition could not account for all of the mycorrhizal fungal response to the altered climatic conditions. Future work should attempt to separate the direct effects of climate change on mycorrhizas from the more indirect effects via vegetation changes. Also, the likelihood that future climate change will result in an altered mycorrhizal fungal community needs to be addressed. However, because of the role of mycorrhizal fungi in the global carbon cycle, how mycorrhizal functioning will be affected by climate change is perhaps the key area to concentrate most research effort.

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References

Augé RM (2001) Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. *Mycorrhiza*, **11**, 3–42.

Begon M, Harper JL, Townsend CR (1990) *Ecology: Individuals, Populations and Communities*, 2nd edn. Blackwell, Oxford.

Buzas MA, Culver SJ (1994) Species pool and dynamics of marine paleocommunities. *Science*, **264**, 1439–1441.

Cao M, Woodward FI (1998) Dynamic responses of terrestrial ecosystem carbon cycling to global climate change. *Nature*, **393**, 249–252.

Casagrandi R, Gatto M (1999) A mesoscale approach to extinction risk in fragmented habitats. *Nature*, **400**, 560–562.

Coleman DC, Crossley DA (1996) *Fundamentals of Soil Ecology*. Academic Press, San Diego.

Davidson EA, Trumbore SE, Amundson R (2000) Soil warming and organic carbon content. *Nature*, **408**, 789–790.

Easterling DR, Meehl GA, Parmesan C *et al.* (2000) Climate extremes: observations, modeling and impacts. *Science*, **289**, 2068–2074.

Fitter AH, Graves JD, Self GK *et al.* (1998) Root production, turnover and respiration under two grassland types along an altitudinal gradient: influence of temperature and solar radiation. *Oecologia*, **114**, 20–30.

Fitter AH, Graves JD, Wolfenden J *et al.* (1997) Root production and turnover and carbon budgets of two contrasting grasslands under ambient and elevated atmospheric carbon dioxide concentrations. *New Phytologist*, **137**, 247–255.

Fitter AH, Heinemeyer A, Staddon PL (2000) The impact of elevated CO_2 and global climate change on arbuscular mycorrhizas: a mycocentric approach. *New Phytologist*, **147**, 179–187.

Grime JP, Brown VK, Thompson K *et al.* (2000) The response of two contrasting limestone grasslands to simulated climate change. *Science*, **289**, 762–765.

Grime JP, Shacklock JML, Band SR (1985) Nuclear DNA contents, shoot phenology and species co-existence in a limestone grassland community. *New Phytologist*, **100**, 435–445.

Harley JL, Harley EL (1987) A check-list of mycorrhiza in the British flora. *New Phytologist* (Suppl), **105**, 1–102.

Harte J, Shaw R (1995) Shifting dominance within a montane vegetation community – results of a climate-warming experiment. *Science*, **267**, 876–880.

van der Heijden MGA, Klironomos JN, Ursic M *et al.* (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, **396**, 69–72.

Hetrick BAD (1991) Mycorrhizas and root architecture. *Experientia*, **47**, 355–362.

Houghton JT, Meira Filho LG, Bruce J *et al.* (1995) *Climate Change 1995: the Science of Climate Change. Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge.

Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytologist*, **115**, 77–83.

Kabir Z, O'Halloran IP, Fyles JW *et al.* (1997) Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: hyphal density and mycorrhizal root colonization. *Plant and Soil*, **192**, 285–293.

Kerr RA (2001) It's official: humans are behind most of global warming. *Science*, **291**, 566.

Killham K (1994) *Soil Ecology*. Cambridge University Press, Cambridge.

Luo Y, Wan S, Hui D *et al.* (2001) Acclimatization of soil respiration to warming in a tall grass prairie. *Nature*, **413**, 622–625.

McGonigle TP, Miller MH, Evans DG *et al.* (1990) A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. *New Phytologist*, **115**, 495–501.

Merryweather JW, Fitter AH (1991) A modified method for elucidating the structure of the fungal partner in vesicular–arbuscular mycorrhiza. *Mycological Research*, **95**, 1435–1437.

Miller RM, Reinhardt DR, Jastrow JD (1995) External hyphal production of vesicular–arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia*, **103**, 17–23.

Newsham KK, Fitter AH, Watkinson AR (1995) Multifunctionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution*, **10**, 407–411.

Nicolson TH (1959) Mycorrhiza in the Gramineae. I. Vesicular–arbuscular endophytes, with special reference to the external phase. *Transactions of the British Mycological Society*, **42**, 421–438.

Oechel WC, Vourtilis GL, Hastings SJ *et al.* (2000) Acclimation of ecosystem CO_2 exchange in the Alaskan Arctic in response to decadal climate warming. *Nature*, **406**, 978–981.

Read DJ (1992) The mycorrhizal mycelium. In: *Mycorrhizal Functioning* (ed. Allen MF), pp. 102–133. Chapman & Hall, New York.

Rillig MC, Wright SF, Shaw MR *et al.* (2002) Artificial climate warming positively affects arbuscular mycorrhizae but decreases soil aggregate water stability in an annual grassland. *Oikos*, **97**, 52–58.

Schimel DS (1995) Terrestrial ecosystems and the carbon cycle. *Global Change Biology*, **1**, 77–91.

Schweiger PF, Jakobsen I (1999) Direct measurement of arbuscular mycorrhizal phosphorus uptake into field-grown winter wheat. *Agronomy Journal*, **91**, 998–1002.

Smith SE, Read DJ (1997) *Mycorrhizal Symbiosis*, 2nd edn. Academic Press, San Diego.

Smith FA, Smith SE (1996) Mutualism and parasitism: diversity in function and structure in the 'arbuscular' (VA) mycorrhizal symbiosis. *Advances in Botanical Research*, **22**, 1–43.

Staddon PL (1998) Insights into mycorrhizal colonisation at elevated CO_2: a simple carbon partitioning model. *Plant and Soil*, **205**, 171–180.

Staddon PL, Fitter AH (1998) Does elevated atmospheric carbon dioxide affect arbuscular mycorrhizas? *Trends in Ecology and Evolution*, **13**, 455–458.

Staddon PL, Fitter AH (2001) The differential vitality of intraradical mycorrhizal structures and its implications. *Soil Biology and Biochemistry*, **33**, 129–132.

Staddon PL, Fitter AH, Graves JD (1999) Effect of elevated atmospheric CO_2 on mycorrhizal colonisation, external hyphal production and phosphorus inflow in *Plantago lanceolata* and *Trifolium repens* in association with the arbuscular mycorrhizal fungus *Glomus mosseae*. *Global Change Biology*, **5**, 347–358.

Staddon PL, Graves JD, Fitter AH (1998) Effect of enhanced atmospheric CO_2 on mycorrhizal colonisation by *Glomus mosseae* in *Plantago Lanceolata and Trifolium repens*. *New Phytologist*, **139**, 571–580.

Tennant D (1975) A test of a modified line intersect method of estimating root length. *Journal of Ecology*, **63**, 995–1001.

Tester M, Smith SE, Smith FA *et al.* (1986) Effects of photon irradiance on the growth of shoots and roots, on the rate of initiation of mycorrhizal infection units in *Trifolium subterraneum* L. *New Phytologist*, **103**, 375–390.

Wyman RL (1991) *Global Climate Change and Life on Earth*. Chapman & Hall, New York.