

# Carbon addition alters vegetation composition on ex-arable fields

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1. Recent changes in European agricultural policy have led to measures to reverse the loss of species-rich grasslands through the creation of new areas on ex-arable land. Ex-arable soils are often characterized by high inorganic nitrogen (N) levels, which lead to the rapid establishment of annual and fast-growing perennial species during the initial phase of habitat creation. The addition of carbon (C) to the soil has been suggested as a countermeasure to reduce plant-available N and alter competitive interactions among plant species.
2. To test the effect of C addition on habitat creation on ex-arable land, an experiment was set up on two recently abandoned fields in Switzerland and on two 6-year-old restoration sites in the UK. Carbon was added as a mixture of either sugar and sawdust or wood chips and sawdust during a period of 2 years. The effects of C addition on soil parameters and vegetation composition were assessed during the period of C additions and 1 year thereafter.
3. Soil nitrate concentrations were reduced at all sites within weeks of the first C addition, and remained low until cessation of the C additions. The overall effect of C addition on vegetation was a reduction in above-ground biomass and cover. At the Swiss sites, the addition of sugar and sawdust led to a relative increase in legume and forb cover and to a decrease in grass cover. The soil N availability, composition of soil micro-organisms and vegetation characteristics continued to be affected after cessation of C additions.
4. *Synthesis and applications.* The results suggest that C addition in grassland restoration is a useful management method to reduce N availability on ex-arable land. Carbon addition alters the vegetation composition by creating gaps in the vegetation that facilitates the establishment of late-seral plant species, and is most effective when started immediately after the abandonment of arable fields and applied over several years.

*Key-words:* C addition, grassland restoration, habitat creation, microbial immobilization, N-enriched ex-arable soil

## Introduction

In the second half of the 20th century, intensification of agricultural management led to a reduction in the extent of species-rich grasslands throughout Europe, through the increased use of inorganic fertilizers and

biocides and the conversion of land to arable cropping. As a response, specific mitigation efforts have been introduced in the European Union (EU) through agri-environment schemes that aim to promote both the extensification of the remaining grassland and creation of species-rich grassland on ex-arable land (Anonymous 1998). Although widely practised, the outcome of such schemes is highly variable and remains difficult to predict (Buckingham *et al.* 1999; Kleijn & Sutherland 2003). This has been attributed to a number of factors,

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such as the lack of seed propagules (Hutchings & Booth 1996; Bekker *et al.* 1997), herbivory (Kleijn 2003) and a paucity of trophic linkages (Van der Heijden 2004).

One of the most critical factors determining the success of creation of species-rich grasslands on ex-arable land is the high nutrient availability resulting from previous land use (Marrs 1993). In habitat-creation projects on fertile soils, a rapid establishment of annual and fast-growing perennial early seral plants often characterizes the initial phase of vegetation development (Hansson & Fogelfors 1998; Baer *et al.* 2004). These continue to dominate as long as the nutrient availability remains high, and thus impede the establishment of late-seral plant species, even when such species are introduced by sowing (Kindscher & Tieszen 1998). Negative effects of high nitrogen (N) availability on the restoration and maintenance of species-rich grassland have been shown in experiments where N availability was increased by fertilization (Tilman 1993; Hansson & Fogelfors 1998). These results suggest that a key prerequisite for succession towards more diverse vegetation on ex-arable land is a reduction in plant-available N (Marrs 1993; Tilman 1993).

Different methods have been proposed to reduce the nutrient availability in ex-arable soils undergoing restoration management (Marrs 1993). These include topsoil removal, maximizing offtake and increasing storage in organic and inorganic nutrient pools. Addition of carbon (C) to the soil has recently been put forward as a means to reduce plant-available nutrients and alter competitive interactions among plant species (Morgan 1994). It has been hypothesized that C addition induces soil microbial activity, which would be paralleled by increased immobilization of inorganic N (Johnson & Edwards 1979; Schmidt, Michelsen & Jonasson 1997; Paschke, McLendon & Redente 2000; Blumenthal, Jordan & Russelle 2003). Several studies have found that C addition leads to decreased rates of net N mineralization (Johnson & Edwards 1979; Averett *et al.* 2004; Gilliam *et al.* 2005) and nitrification (Gilliam *et al.* 2005) and reduced concentrations of ammonium ( $\text{NH}_4^+$ ; Hopkins 1998) and nitrate ( $\text{NO}_3^-$ ; Schmidt, Michelsen & Jonasson 1997; Török *et al.* 2000; Blumenthal, Jordan & Russelle 2003) in the soil. As plant growth is thought to be primarily limited by the availability of inorganic N in the soil (Tilman 1985; Schimel & Bennett 2004), a reduction in the amount of plant-available N in response to C addition should result in reduced plant growth (Blumenthal, Jordan & Russelle 2003). While this method has been tested repeatedly to reduce the competitive ability of alien invasive plants (Reever Morghan & Seastedt 1999; Alpert & Maron 2000; Blumenthal, Jordan & Russelle 2003; Corbin & d'Antonio 2004; Perry, Galatowitsch & Rosen 2004) it has rarely been considered as a tool to alter the community composition of native vegetation (Michelsen *et al.* 1999; Török *et al.* 2000; Eschen, Müller-Schärer & Schaffner 2006). C addition has also been found to reduce above-ground vegetation biomass (Michelsen

*et al.* 1999; Alpert & Maron 2000; Blumenthal, Jordan & Russelle 2003). In nutrient-rich environments, such as ex-arable soils,  $\text{NO}_3^-$  is expected to be the dominant pool of plant-available N (Schimel & Bennett 2004); it might therefore be expected that a reduction in  $\text{NO}_3^-$  through C addition will affect the growth rate of plants on ex-arable land.

The type of C source added to the soil is likely to influence the effect of C addition on the soil environment and plant growth as a result of the rate at which the source is available to micro-organisms. A readily available C source such as sugar (sucrose) may stimulate microbial activity within hours (Dalenberg & Jager 1981) while other sources, consisting of structurally more complex molecules, take longer to degrade (Magill & Aber 2000), especially when applied as coarse structures with small surface to volume ratios. The decay of sawdust is slower than that of sugar (Török *et al.* 2000) but presumably faster than that of wood chips. The relatively short decay rate of sawdust makes it a potential substitute for the expensive sugar, while the addition of small pieces of wood may have a slower but longer-lasting effect on the soil environment and vegetation.

The increase in microbial N immobilization through C addition may be a temporary phenomenon. Little is known about how the soil microbial community and the pool of plant-available N in C-amended soil responds to a cessation of C addition, and how these effects will translate to a shift in vegetation composition. Once C addition is stopped, the inorganic N concentration in the soil may rise again as a result of an increased release from decaying microbial biomass (Török *et al.* 2000). The rate of release of stored N may affect the extent to which C addition will influence soil N concentration and the vegetation composition after the period of C addition. The timing and rate of such a release may depend on the group of micro-organisms involved in the storage of nutrients. The addition of C as sugar may elicit a quick response in terms of bacterial biomass, while addition of C as wood chips may particularly stimulate the activity of decomposer fungi because of their ability to exploit larger structures. The release from bacterial biomass is likely to be faster than from groups with slower turnover rates, such as fungi. As we expect wood chips to stimulate decomposing fungi in particular, the release of N in soils where wood chips were added may occur later and at a lower rate than in soils where sugar was added.

The general effect of C addition on the vegetation is a reduction in plant growth, resulting in reduced cover and competition for light (Blumenthal, Jordan & Russelle 2003 and references therein). Yet to make C addition a useful tool to manage vegetation composition in habitat-creation projects, it should affect the growth of plant species in a species-specific way. In a greenhouse experiment testing the effect of C addition on the growth of a large number of early seral and late-seral plant species in the absence of competition, Eschen,

Müller-Schärer & Schaffner (2006) found that C addition reduced the growth of all plant species but that the level of biomass reduction varied significantly among the plant species. Both functional group and life form explained significant amounts of the variation among individual plant species. For example, biomass reduction in legumes was lower than that in other forbs and grasses, and the shoot:root ratios of grasses were significantly reduced while those of legumes and other forbs were not. Moreover, the growth of annual plants was affected more by C addition than that of perennial plant species. These findings are in line with the results of a study by Averett *et al.* (2004) and suggest that C addition may indeed be a promising tool for manipulating vegetation composition on N-rich ex-arable land.

In the present study we aimed to assess the effect of different forms of C addition on vegetation composition at ex-arable sites. We examined the effect of C addition on the soil nutrient availability, soil microbial community and vegetation composition at two recently abandoned fields in Switzerland and two 6-year-old restoration sites in the UK. We monitored changes in these factors during a 2-year period of C addition and the year following cessation of the treatments. We tested the hypotheses that: (i) C addition leads to a reduction in plant-available N in the soil; (ii) this reduction coincides with an increase in microbial biomass; (iii) the changes in the soil environment lead to changes in plant above-ground biomass and changes in vegetation composition; (iv) the changes in vegetation cover facilitate the establishment of sown late-seral plant species; (v) the magnitude of the effect during the period of C addition depends on the form of the C source applied; and (vi) the effects will persist for at least 1 year beyond the period of C addition.

## Methods

### SITES

The experiment was set up on four ex-arable fields, two each in Switzerland and the UK. The two Swiss field sites, which were located in the Jura mountains near the villages of Movelier and Courchapoix, were *c.* 0.3 ha in extent and had been used for arable cultivation of wheat in the season prior to the experiment. The Move-

lier site was on a south-east-facing slope on a regosol soil on Jurassic chalk bedrock. The Courchapoix site was located on a north-west-facing slope on a gley soil on Jurassic chalk bedrock. The mean temperature in January is  $-0.4$  °C (mean minimum  $-3.4$  °C, maximum  $2.4$  °C), mean temperature in July  $17.0$  °C (mean minimum  $11.9$  °C, maximum  $22.7$  °C) and mean annual precipitation 903 mm (climate data from the closest weather station, 10–15 km away from the experimental sites, 30-year averages). At the start of the experiment in May 2002, the fields were ploughed and chiselled. In August 2002, a seedbed was prepared by harrowing and the experimental plots were sown by hand with a 10-species seed mixture comprising the characteristic late-seral grassland plant species *Festuca rubra* L. s.l., *Poa pratensis* L., *Cynosurus cristatus* L., *Holcus lanatus* L., *Plantago lanceolata* L., *Centaurea jacea* L. s.l., *Leontodon hispidus* L. s.l., *Sanguisorba minor* Scop. s.l., *Trifolium pratense* L. s.l., and *Trifolium dubium* Sibth. Sowing densities were 500 seeds  $m^{-2}$  for grasses and 100 seeds  $m^{-2}$  for forbs and legumes. Seeds were obtained from a local commercial seed supplier. The sites were mown once a year after 15 June using a bar mower and the hay was removed.

The experimental sites in the UK were located on *c.* 6.0- and 7.5-ha, adjacent ex-arable fields in Oxfordshire. One site was located on a north-west-facing slope while the other was located in the bottom of the same valley. The mean temperature in January is  $3.4$  °C (mean minimum  $0.6$  °C, maximum  $6.0$  °C), mean temperature in July  $16.0$  °C (mean minimum  $10.9$  °C, maximum  $21.0$  °C) and the annual precipitation 750 mm (climate data from the weather station at Oxford, adjusted to local conditions, 30-year averages). The soil at the sites is a grey rendzina on Cretaceous chalk bedrock. The fields were taken out of arable cultivation in 1996 and were in set-aside for 5 years before entering conservation management in 2001, and have been left to be colonized naturally. A species-rich chalk grassland, which is part of the Aston Rowant National Nature Reserve, was located adjacent to the experimental sites. The ex-arable fields and the species-rich grassland were grazed by sheep. The ex-arable field was not sown prior to the experiment. Selected characteristics of each of the four field sites, including initial inorganic N availability, are summarized in Table 1.

**Table 1.** Characteristics of the four study sites. Samples for measurement of initial soil chemical conditions were taken just before the first C addition. Initial values for soil chemistry are given as mean  $\pm$  SE

Site	Coordinates	Field preparation	Vegetation age (years)	Management	N-NO <sub>3</sub> <sup>-</sup> (p.p.m.)	N-NH <sub>4</sub> <sup>+</sup> (p.p.m.)	pH
Switzerland							
Movelier	7°19'E, 47°25'N	Harrowed, sown	0	Mowing	31.11 $\pm$ 2.97	10.18 $\pm$ 0.61	7.57 $\pm$ 0.04
Courchapoix	7°26'E, 47°21'N	Harrowed, sown	0	Mowing	51.66 $\pm$ 3.10	6.50 $\pm$ 0.38	7.40 $\pm$ 0.02
UK							
Slope	0°56'W, 51°39'N	Not prepared	6	Sheep grazing	17.73 $\pm$ 0.88	6.80 $\pm$ 0.37	7.72 $\pm$ 0.02
Valley	0°56'W, 51°39'N	Not prepared	6	Sheep grazing	42.38 $\pm$ 3.64	7.72 $\pm$ 0.27	7.71 $\pm$ 0.01

## EXPERIMENTAL SET-UP

At each site, plots were laid out in three rows, parallel to the adjacent grassland, each consisting of three blocks. The row nearest to the existing grassland was 2–5 m inside the ex-arable land and the others were 15 m and 30 m from this row. The blocks consisted of three experimental plots, each of which measured 2 × 3 m, with 0.5-m paths between the plots. The design gave a total of 54 plots per country. Three treatments were randomly assigned to the plots within each block. The treatments were: (i) a mixture of sawdust and sugar (0.22 kg C m<sup>-2</sup> application<sup>-1</sup>, sawdust-to-sugar ratio 1:1 based on C content); (ii) a mixture of sawdust and wood chips (0.22 kg C m<sup>-2</sup> application<sup>-1</sup>, sawdust-to-wood chips ratio 2:1 based on C content); and (iii) no C addition (control). The sawdust and wood chips, derived from native broadleaf species, were obtained from local sawmills and were air-dried before application. Sugar, sucrose made from sugar beet, was obtained from local commercial sources.

Treatments were applied by hand three times in autumn 2002 (late August, mid-September, early October) and twice in spring 2003 (early March, mid-April). No wood chips were applied in 2003 as previous applications degraded only slowly and accumulated on the soil surface. Sugar and sawdust treatments were stopped after April 2003. At that time, a thin cover of undegraded sawdust was present on the soil surface of the UK sites while the sawdust was almost completely decomposed at the Swiss sites. The combined applications resulted in a total addition of approximately 1.1 kg C m<sup>-2</sup> and 0.95 kg C m<sup>-2</sup> for the sawdust and sugar, and sawdust and wood chips, treatments, respectively.

## MEASUREMENTS

Soil samples, for analysis of available N and soil microbial community composition, were taken from the three plots of the middle row at each site (a total of 18 samples per sampling and country). Samples were taken three times in late summer 2002, three times in spring 2003, once in autumn 2003 and three times in spring 2004. The five first samplings coincided with the application of C treatments; soil samples were taken on the same day, before adding C. At each sampling date, five soil cores (5 cm diameter, 10 cm depth) were taken per plot and pooled for analysis. Samples were stored at -20 °C until analysis.

The soil samples were air-dried (< 30 °C), sieved (< 2 mm) and analysed for inorganic N (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) content. Inorganic N was extracted by shaking 10 g soil in 30 mL 1 M KCl and filtered (Whatman no. 42). NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations in the extracts were then determined in a Brau & Luebe autoanalyser mod (Brau & Luebe, Hamburg, Germany). 3 equipped with an AA3 digital colorimeter (Brau & Luebe, Hamburg, Germany) following the manufacturer's instructions.

The soil microbial community composition was analysed in subsamples of the soil samples taken for the

analysis of soil chemistry. The samples taken in 2002, spring 2003 and 2004 were analysed for the Swiss sites (a total of nine dates) and the samples taken in 2002 and 2003 for the UK sites (total seven samples). Organic matter content was determined gravimetrically by weighing the soil before and after burning at 400 °C for 8 h. Soil microbial community composition was estimated using the phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) techniques (Frostegård, Bååth & Tunlid 1993). PLFA i15:0, a15:0, 15:0, i16:0, 16:1ω9, i17:0, a17:0, cy17:0, 18:1ω7 and cy19:0 were chosen to represent bacterial biomass; PLFA 18:6ω2 represented saprophytic fungal biomass and NLFA 16:1ω5 represented arbuscular mycorrhizal (AM) fungi (Hedlund 2002). The amounts of PLFA and NLFA were expressed as nmol g<sup>-1</sup> soil.

Plant above-ground biomass was sampled once per year as close to peak standing biomass as possible in 2002, 2003 and 2004. The biomass was cut from a 25 × 50-cm area within the 0.5-m border strip of each plot, dried at 60 °C for 2 days and weighed. Vegetation composition was assessed in the central 1-m<sup>2</sup> quadrat in each plot by visually estimating the cover of each vascular species to the nearest per cent, and the cover of the three functional plant groups grasses, legumes and other forbs was determined by summing the cover of each vascular plant species belonging to each of the functional groups. In addition, the total cover of bryophytes and bare ground in the central 1-m<sup>2</sup> quadrat were estimated to the nearest per cent. Vegetation was monitored once in 2003 and 2004. Bare ground estimation was not standardized in the two countries, which resulted in different mean estimates; we therefore only present differences between treatments.

## STATISTICAL ANALYSIS

The results from the Swiss and UK sites were analysed separately because of the differences in age, management of the ex-arable fields and stage of vegetation development (Table 1). Data from the period when treatments were applied (until 6 weeks after the last C addition) and the period thereafter were analysed separately to distinguish between responses caused during the treatments and effects persisting beyond the period of C addition.

Effects of C addition on soil NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> content, and estimated biomass of bacteria, AM fungi, saprophytic fungi and fungal:bacterial ratio, were analysed using general linear models, with treatment, site and time as fixed factors and blocks nested within site as a random factor. Treatment effects were tested against treatment by block by site interaction and time was tested against residuals. Differences among treatments within sampling dates were tested using general linear models, with treatment, site and time as fixed factors and blocks nested within site as a random factor. Above-ground vegetation biomass and cover of grasses, legumes, non-leguminous forbs and bryophytes, as percentages of total vegetation cover, were analysed using general

linear models, with treatment and site as fixed factors and block nested within site as a random factor. Mean values of response variables in the treatments were compared using Tukey HSD. *P*-values smaller than 0.05 were considered significant. Changes in soil NO<sub>3</sub><sup>-</sup> and biomass of bacteria, AM fungi and saprophytic fungi between the first and second sampling dates were calculated for each plot and analysed using linear regression.

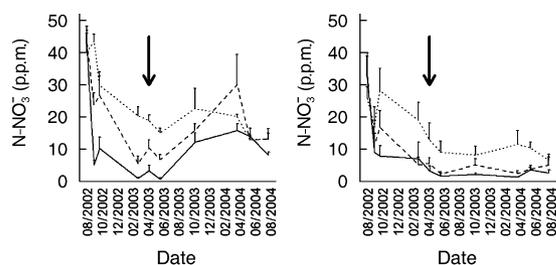
General linear models and Tukey HSD were calculated with SPSS for Windows (version 10). The other analyses were calculated with the R statistics package version 1.8.1 for Windows using vegan package version 1.6-7 (R Development Core Team 2004).

## Results

### INORGANIC N AVAILABILITY

At the Swiss sites, the NO<sub>3</sub><sup>-</sup> concentration was significantly affected by C addition during the period of application ( $F_{2,8} = 99.271$ ,  $P < 0.001$ ). The NO<sub>3</sub><sup>-</sup> concentration decreased more quickly and remained lower in sugar/sawdust-amended plots than in control plots, with wood chip/sawdust-amended plots showing an intermediate response (Fig. 1). After C addition was stopped, the NO<sub>3</sub><sup>-</sup> concentration in C-amended plots increased but remained lowest on the sugar/sawdust-amended plots ( $F_{2,8} = 4.946$ ,  $P < 0.05$ ). The NH<sub>4</sub><sup>+</sup> concentration was not affected by the treatments during the period of application ( $F_{2,8} = 0.522$ ,  $P = 0.612$ ). After the period of C addition, a significant treatment by time interaction was found ( $F_{2,8} = 4.738$ ,  $P < 0.05$ ) but mean values differed only in March 2004 ( $F_{2,8} = 7.696$ ,  $P < 0.05$ ), when the NH<sub>4</sub><sup>+</sup> concentration was higher in wood chip/sawdust-amended plots than in control plots and sugar/sawdust-amended plots (Tukey test  $P < 0.05$ ).

The NO<sub>3</sub><sup>-</sup> concentration at the UK sites was affected by C addition during the period of application in a similar way to that found at the Swiss sites ( $F_{2,8} = 27.228$ ,  $P < 0.001$ ; Fig. 1). However, the NO<sub>3</sub><sup>-</sup> concentration did not increase after the last C addition but continued to be significantly lower in C-amended plots compared with the control plots ( $F_{2,8} = 27.073$ ,  $P < 0.001$ ; Fig. 1).



**Fig. 1.** Mean NO<sub>3</sub><sup>-</sup> levels in the different treatments at the Swiss (left) and UK (right) sites. Solid, dashed and dotted lines indicate means for plots that received sugar and sawdust, wood chips and sawdust, and control plots, respectively. Error bars indicate SE. Vertical arrows indicate the date of the last C additions.

The NH<sub>4</sub><sup>+</sup> concentration was unaffected by C addition throughout the course of the study.

### SOIL MICROBIAL COMMUNITY COMPOSITION AND BIOMASS

At the Swiss sites, the change in NO<sub>3</sub><sup>-</sup> concentration within 4 weeks of the first C addition was negatively correlated with the changes in total bacterial and saprophytic fungal PLFA, as well as with AM fungal NLFA (linear regressions,  $n = 18$ ,  $P < 0.001$ ,  $R^2 = 0.52$ ,  $y = -1.92x - 12.58$ ;  $n = 18$ ,  $P < 0.05$ ,  $R^2 = 0.26$ ,  $y = -14.60x - 19.65$ ;  $n = 18$ ,  $P = 0.010$ ,  $R^2 = 0.39$ ,  $y = -11.24 - 18.24$ , respectively).

Mean abundance of bacterial PLFA at the Swiss sites was affected by the treatments during the period of application ( $F_{2,8} = 5.893$ ,  $P < 0.05$ ), with moderately higher levels in sugar/sawdust-amended plots. No effects of C addition on bacterial PLFA were found after the treatments were stopped ( $F_{2,8} = 3.535$ ,  $P = 0.902$ ; Fig. 2). Abundance of AM fungal NLFA differed significantly between the treatments after C addition was stopped ( $F_{2,8} = 4.954$ ,  $P < 0.05$ ) but no consistent pattern could be detected (Fig. 2).

At the Swiss sites, no overall effect of C addition was found on the fungal:bacterial ratio of C-amended and control plots during the period of C addition. C addition led to an increase in the ratio at the Movelier site, while the ratio at the Courchapoix site decreased (treatment  $\times$  site interaction  $F_{2,8} = 17.92$ ,  $P = 0.001$ ). In March 2003, control plots had a lower fungal:bacterial ratio than C-amended plots but this difference was not found subsequently in spring 2003. After cessation of the treatments, a significant effect of the treatments on the fungal:bacterial ratio was found, with control plots having a consistently lower ratio than wood chip/sawdust-amended plots ( $F_{2,8} = 5.11$ ,  $P < 0.05$ ; Fig. 2).

At the UK sites no correlations were found between changes in biomass and NO<sub>3</sub><sup>-</sup>. Bacterial and saprophytic fungal biomass were affected by the C addition during the period of C application ( $F_{2,8} = 12.160$ ,  $P < 0.005$  and  $F_{2,8} = 12.986$ ,  $P < 0.005$ , respectively). Bacterial biomass was highest in the sugar/sawdust-amended plots, followed by the wood chip/sawdust and then the control plots (Fig. 2). The saprophytic fungal biomass was most affected by the wood chip/sawdust treatment (Fig. 2). Saprophytic fungal biomass still varied among the treatments after C additions were stopped ( $F_{2,8} = 8.406$ ,  $P < 0.05$ ), with control plots having significantly lower biomass than wood chip/sawdust- and sugar/sawdust-amended plots (Fig. 2).

The fungal:bacterial ratio at the UK sites was marginally affected by treatments during the period of addition ( $F_{2,8} = 3.91$ ,  $P = 0.07$ ). The ratio was significantly higher in wood chip/sawdust-amended plots than in control plots (Tukey test  $P < 0.05$ ; Fig. 2). After C addition was stopped, the fungal:bacterial ratio remained higher in wood chip/sawdust-amended plots compared with control plots ( $F_{2,8} = 19.48$ ,  $P < 0.001$ ; Fig. 2).

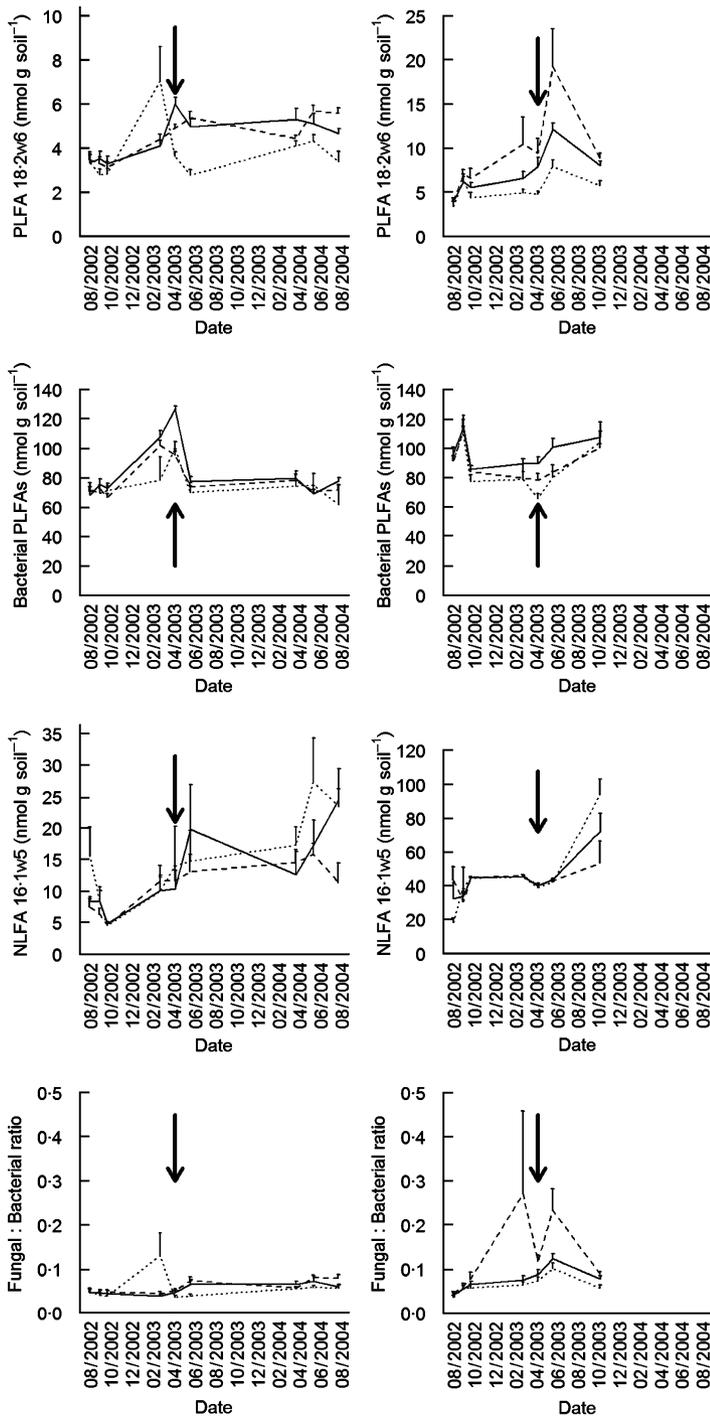


Fig. 2. Mean levels of saprophytic fungal, total bacterial PLFA and AM fungal NLFA, and fungal:bacterial ratios in the different treatments at the Swiss (left) and UK (right) sites. Solid, dashed and dotted lines indicate means for plots that received sugar and sawdust, wood chips and sawdust, and control plots, respectively. Error bars indicate SE. Vertical arrows indicate the date of the last C additions.

#### VEGETATION BIOMASS AND COVER OF FUNCTIONAL GROUPS

At the Swiss sites, vegetation biomass differed significantly among treatments in 2002 ( $F_{2,28} = 14.760$ ,  $P < 0.001$ ). The biomass was lowest in sugar/sawdust-amended plots, followed by wood chip/sawdust plots and control plots (mean  $\pm$  SE;  $0.056 \pm 0.007$ ,  $0.031 \pm 0.005$  and

$0.021 \pm 0.004$  g m<sup>-2</sup>, respectively). In 2002, the peak biomass at the Swiss sites correlated with the soil NO<sub>3</sub><sup>-</sup> concentration 4 weeks after the first C addition (linear regression,  $n = 18$ ,  $P < 0.005$ ,  $R^2 = 0.50$ ). In 2003, the biomass was significantly lower in C-treated plots than in control plots at the UK sites ( $F_{2,28} = 5.000$ ,  $P < 0.05$ ; mean  $\pm$  SE,  $0.185 \pm 0.028$ ,  $0.120 \pm 0.015$  and  $0.117 \pm 0.014$  g m<sup>-2</sup> in control plots, wood chip/sawdust plots and sugar/sawdust plots, respectively) but not at the Swiss sites. In 2004, no differences in vegetation biomass were found between the treatments, at either the Swiss or UK sites.

Both in Switzerland and the UK, only native species were found in the experimental plots. At the Swiss sites, the relative cover of grasses, forbs and legumes was affected by the treatments in 2003 ( $F_{2,32} = 37.895$ ,  $P < 0.001$ ;  $F_{2,32} = 12.124$ ,  $P < 0.001$ ;  $F_{2,32} = 4.620$ ,  $P < 0.05$ , respectively). Adding sugar/sawdust led to a significant reduction in the cover of grasses and to a significant increase in the cover of forbs and legumes (Fig. 3). Bare ground was significantly increased by C addition ( $F_{2,32} = 37.482$ ,  $P < 0.001$ ), with about 14% more bare ground on wood chip/sawdust-amended and 22% on sugar/sawdust-amended plots than on control plots. In 2004, the relative cover of grasses and legumes continued to be affected by the treatments ( $F_{2,32} = 8.958$ ,  $P < 0.001$  and  $F_{2,32} = 8.112$ ,  $P < 0.005$ , respectively). The cover of legumes remained higher and the cover of grasses lower on sugar/sawdust-amended plots than on control plots (Fig. 3). The cover of grasses was negatively correlated with the cover of legumes (linear regression,  $n = 54$ ,  $P < 0.001$ ,  $R^2 = 0.44$ ). Bare ground continued to be significantly affected by the C addition ( $F_{2,32} = 16.027$ ,  $P < 0.001$ ), with about 6% more bare ground on wood chip/sawdust-amended and 9% on sugar/sawdust-amended plots than on control plots.

The relative cover of the sown late-seral species differed between treatments in 2003 ( $F_{2,32} = 6.121$ ,  $P < 0.01$ ), with values higher on sugar/sawdust-amended plots than on control plots. The relative cover of sown forbs was 11% higher and that of sown legumes 6% higher on sugar/sawdust-amended plots, while the relative cover of sown grasses did not differ between sugar/sawdust-amended plots and control plots. The relative cover of sown species increased significantly in response to the treatments at the Courchapoix site, while no changes were found at the Movelier site (treatment by site interaction,  $F_{2,32} = 6.145$ ,  $P < 0.01$ ; Fig. 4). In 2004, the relative cover of sown species did not differ between treatments ( $F_{2,32} = 0.203$ ,  $P = 0.810$ ).

At the UK sites, the cover of bryophytes was affected by the treatments in 2003 ( $F_{2,32} = 6.166$ ,  $P < 0.01$ ). The cover of bryophytes was lower on sugar/sawdust plots than on control plots (Fig. 3). As observed at the Swiss sites, the amount of bare ground at the UK sites was also significantly affected by the treatments ( $F_{2,32} = 9.876$ ,  $P < 0.001$ ) but the differences were small; the wood chip/sawdust-amended plots had 5% more bare ground than sugar/sawdust-amended and control plots. The

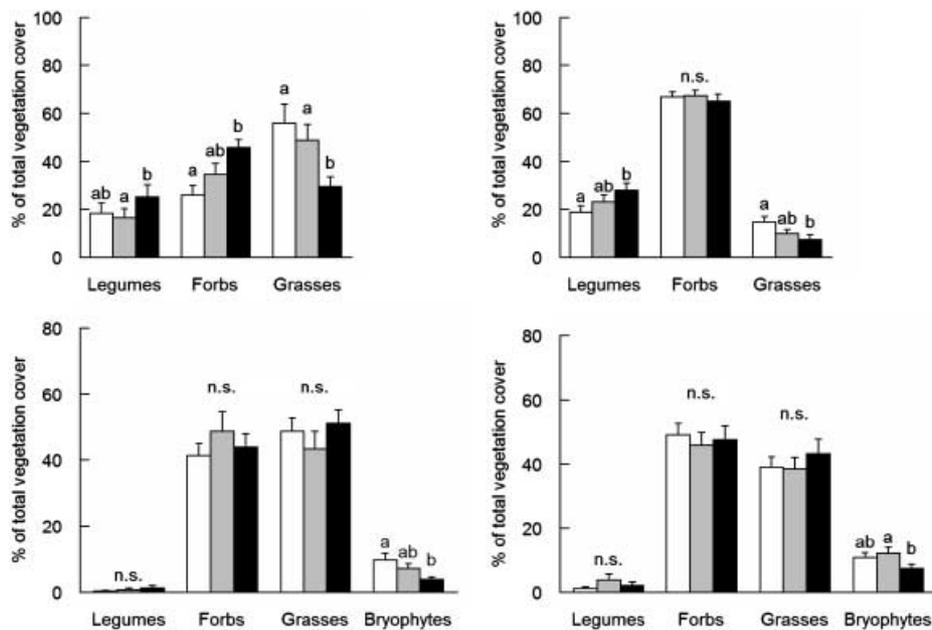


Fig. 3. Relative cover (mean + SE) of different functional plant groups in the different treatments for Swiss (upper figures) and UK (lower figures) sites for 2003 (left) and 2004 (right figures). Bryophyte, grass, legume and forb cover are percentages of total higher plant cover. Characters refer to comparison of treatment means within each year.

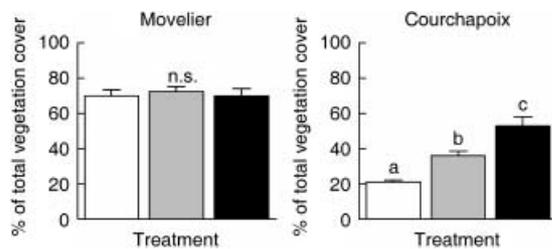


Fig. 4. Relative cover of sown species in the vegetation at the two Swiss sites in 2003. Error bars indicate SE. Characters above bars refer to comparison of mean values (Tukey test,  $P < 0.05$ ).

cover of bryophytes continued to be affected by the treatments in 2004 ( $F_{2,32} = 3.382$ ,  $P < 0.05$ ) and cover was lowest on sugar/sawdust-amended plots (Fig. 3). Likewise, bare ground also differed among treatments in 2004 ( $F_{2,32} = 8.802$ ,  $P < 0.005$ ) and was 5% higher on wood chip/sawdust-amended and 7% higher on sugar/sawdust-amended plots than on control plots.

## Discussion

The results of the present study confirm that C addition can significantly reduce the availability of inorganic N in the soil. The  $\text{NO}_3^-$  concentration was reduced within 4 weeks of the first C addition and remained lower than in the control plots throughout the period of treatment application (Fig. 1). The response of  $\text{NO}_3^-$  to C addition and the correlated changes in microbial biomass suggest that C was the limiting resource for microbial activity at the Swiss sites, and that the decrease in inorganic N was mediated by the micro-organisms. These findings are consistent with previous studies

documenting an increase in net N immobilization by soil microbes after C addition (Johnson & Edwards 1979; Magill & Aber 2000). In our study, the reduction in plant growth that coincided with the reduction in  $\text{NO}_3^-$  indicated that  $\text{NO}_3^-$  was the limiting nutrient for plant growth (Schimel & Bennett 2004). The mean biomass of bacteria showed only a moderate increase in C-amended soil during the period of C addition, while the biomass of saprophytic fungi was higher on C-amended soil only in the UK. The relatively low differences in mean biomass of bacteria between C-amended and control plots may be explained either by an increase in the N concentration of microbial biomass, rather than an increase in abundance (Michelsen *et al.* 1999; Corbin & d'Antonio 2004), or by a parallel increase in bacteria-feeding organisms that regulate bacterial abundance (Mikola 1998).

AM fungi take up C from the soil before they associate with a host-plant (Bago *et al.* 1999), which may explain the correlation between the change in  $\text{NO}_3^-$  concentration and change in AM fungal biomass in the weeks after the first C addition at the Swiss sites. An increase in C availability after C addition, as in this study, may therefore increase the rate of  $\text{NO}_3^-$  consumption and activity of AM fungi for a short period of time. AM fungal growth is no longer affected by soil C once associations have been formed with a host-plant, which may explain why we did not find a consistent effect of C addition on AM fungal abundance. The significant treatment effect on AM fungal biomass at the Swiss sites after the C additions were stopped was because of differences on one sampling date only.

In general, the pattern of N availability at the UK sites was similar to that at the Swiss sites (Fig. 1); however,

there were no treatment effects on  $\text{NO}_3^-$  during the first weeks of C addition, which may explain why no relationship between changes in N availability and changes in microbial biomass was found at the UK sites. The slower response of the soil at the UK sites may be a result of the extended period of set-aside of the fields prior to the start of the experiment, which may have resulted in the establishment of below-ground and above-ground communities that make the system more resilient to changes in resource availability.

The magnitude of the reduction in  $\text{NO}_3^-$  in the present study depended on the C source. The sugar/sawdust mixture elicited faster and stronger changes in the  $\text{NO}_3^-$  concentration, and the vegetation parameters we measured, than the wood chip/sawdust mixture (Figs 1 and 3). The faster response in sugar/sawdust plots than in wood chip/sawdust plots is likely to be because of the sugar, which is more easily available to microorganisms than sawdust and can thus elicit a quicker reduction in  $\text{NO}_3^-$  concentration, than structurally more complex C sources (Török *et al.* 2000). Similarly, Johnson & Edwards (1979) found that adding sucrose increased net N immobilization more rapidly than adding less-labile C sources such as root and litter leachates. The release of C from wood chips may have been slower because of the coarser structure of wood chips compared with sawdust. In addition, the wood chip/sawdust plots received less C than the sugar/sawdust plots, because no wood chips were added in 2003. The rate of N immobilization by the soil microbial community has been found to increase logarithmically in response to C addition (Gilliam *et al.* 2005). Similarly, plant growth responses have been shown to depend on the amount of C added to the soil (Blumenthal, Jordan & Russelle 2003; Eschen, Müller-Schärer & Schaffner 2006). In our study, the difference in magnitude of the responses between the two C treatments was already apparent in 2002, indicating that the results are best explained by the different availability of the added C.

The changes in saprophytic fungi were paralleled by an increase in the fungal:bacterial ratio in wood chip/sawdust plots in both countries. The fact that the wood chip/sawdust treatment induced a biomass increase of saprophytic fungi at the UK sites and at one of the two Swiss sites provides evidence that the wood chips were available to fungi as a C source. The shift in the fungal:bacterial ratio in the soil of wood chip/sawdust plots lasted beyond the period of wood chip addition (Fig. 3), which is likely to be a consequence of the slow degradation of the wood chips; wood chips were still detectable on the soil surface at the end of the study. The present results suggest that it may be possible to manipulate below-ground microbial succession on ex-arable fields differently by adding either slowly or rapidly decomposing C sources to the soil.

In a greenhouse study, native European grassland species, when grown without competition, showed species-specific reductions in growth in response to C addition (Eschen, Müller-Schärer & Schaffner 2006).

The above-ground biomass of annual plant species and grasses was significantly more reduced than that of legumes and perennial forbs. In the present field study, the changes in cover of the different plant functional groups during the period of C addition support the findings of the greenhouse study and show that different growth rates after C addition can have a significant impact on the composition of the vegetation. Similarly, in a study on ex-arable land invaded by exotic species, Averett *et al.* (2004) found that the reduction in above-ground biomass after C addition was less pronounced for prairie forbs compared with prairie grasses and exotic species. These and other studies documenting a change in the vegetation composition after C addition (Michelsen *et al.* 1999; Blumenthal, Jordan & Russelle 2003; Perry, Galatowitsch & Rosen 2004) demonstrate that C addition is a promising tool for manipulation of vegetation composition in disturbed habitats.

The Swiss and the UK sites differed in several aspects at the start of the experiment, such as time since abandonment and management (Table 1). Therefore the different responses of the plant functional groups to C addition at the UK and Swiss sites cannot be compared directly. One potential explanation for the observed differences is that the age of the vegetation at the start of the treatment application varied between the sites. The seedlings colonizing the Swiss sites were most probably free or almost free of mycorrhizae during the first days and weeks after germination, and were therefore likely to be more susceptible to changes in soil inorganic nutrient content than the established plants at the UK sites, the majority of which belonged to mycorrhizal plant species. Mycorrhizae are known to act as a support system to plant growth because they facilitate nutrient uptake by the plants (Schimel & Bennett 2004). We therefore hypothesize that C addition has the strongest impact on the vegetation composition on ex-arable land when applied immediately after taking the land out of cultivation.

The higher level of bare ground on C-amended plots at the Swiss sites was paralleled by a reduction in the cover of unsown, spontaneously occurring plant species. It appears that the creation of open gaps enabled better establishment of sown species during the period of C addition at the Swiss site in Courchapoix, which was characterized by a high abundance of unsown grasses in the control plots (Fig. 4). In the UK, the abundance of bryophytes, a group absent at the Swiss sites, was reduced, creating more bare patches on those sites. Tilman (1993) suggested that increased light penetration promotes the rate of establishment by late-seral species in grasslands. The promotion of open patches at the beginning of grassland development, as observed in this experiment, shows the potential of C addition to create conditions that are desirable for the creation of species-rich grasslands. Moreover, the increase in abundance of the sown species at the Swiss site in Courchapoix, where the abundance of unsown grasses was high compared with the level found at the other Swiss site at Movelier,

indicates that C addition can make the outcome of restoration programmes more predictable.

The results from the Swiss sites show that C addition can create gaps in the vegetation and may thus increase the possibility for late-seral species to establish over an extended period of time. Whether the persistence of open space is a desirable outcome or not depends on the identity of the colonizing species. At the Swiss sites the bare patches that were present when C addition was stopped in summer 2003 were colonized by annual forbs in 2004. Thus, although in 2004 the cover of legumes remained significantly higher and the cover of grasses lower on the sugar/sawdust-amended plots than on the control plots, the vegetation composition on the C-amended plots had already started to approach that of the control plots. We suggest that C addition should be continued until the gaps in the vegetation have been more or less fully colonized by desirable plant species. This may be attained by either adding smaller amounts of C, which would reduce the level of growth reduction, or applying C over a longer period than in this experiment.

In summary, our study provides evidence that C addition is a useful tool that could be used to reduce N availability in ex-arable soils, thereby increasing the likelihood of successful creation of species-rich grassland. The rapid response and the low cost of C addition make it a useful alternative to other methods for reducing soil nutrient availability (Marrs 1993), such as topsoil removal and hay cutting, which are more expensive or have slower effects on nutrient availability. We therefore support the recommendation for management of ex-arable fields on fertile soils made by Hansson & Fogelfors (1998), that the initial dominance of early seral species can be reduced and diversity of the vegetation increased by adding C to the soil. C addition could be combined with other management tactics, such as sowing a species-rich seed mixture or cutting to increase species richness.

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