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Vegetation, soils and seed banks of limestone grasslands are still impacted by former cultivation one century after abandonment

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Abstract: This study identifies the long lasting impacts of former cultivation on soils, seed banks and above-ground vegetation of limestone grasslands. We compared the resilience of three crop fields cultivated in the 19th century and abandoned (Abandoned Fields) with three grasslands which have never been cultivated (Old Grasslands). Grasslands were located in the Nature reserve of Grand-Pierre and Vitain valleys in France. Sites were identified using historical sources. Chemical and physical soil properties, above-ground vegetation and soil seed bank (0-10 cm and 10-20 cm) were studied. Data were analysed using a multivariate and univariate analyses to detect the effects/impacts of ancient cultivation. Our results clearly show that soil properties (e.g., calcium, carbonate, clay contents), above-ground vegetation (species diversity, moss and lichen cover) and seed bank (floristic composition, species-richness and diversity) are still impacted more than one century after their abandonment. Species richness of both above ground vegetation and seed bank are higher in old grasslands than in formerly cultivated fields. In the seed bank of the formerly cultivated soils we also found the presence of a very rare arable weed species (*Althaea hirsuta*) which has not been inventoried for a long time in the above-ground vegetation of the nature reserve. The resilience of formerly cultivated limestone grasslands might be influenced by the present management regime (site effect). Nevertheless, the resilience period of limestone grasslands is very long—more than one century—and return to an initial state might be difficult or impossible to reach.

Nomenclature: Kerguelen (1999).

Abbreviations: AF—Abandoned Fields, OG—Old Grassland.

Introduction

Understanding ecosystem stability and the response of ecosystems to disturbance is essential for conservation management and successful restoration (Temperton et al. 2004). This is particularly true in regularly disturbed plant communities with a high conservation value (Hirst et al. 2003, 2005). In north-western Europe, calcareous grasslands are such habitats. These species-rich grasslands on calcareous soils are plant communities that have been maintained open by human disturbance (e.g., grazing, mowing, and burning) for many centuries (Sutherland and Hill 1995, Dutoit et al. 2009). Within the last few decades, agricultural intensification has led to considerable floristic changes and to a decrease in calcareous grasslands (WalliesDeVries et al. 2002). Many formerly continuous types of calcareous grasslands have become extinct or fragmented, and only small patches are left (Muller et al. 1998, Poschlod et al. 1998). The protection of calcareous grasslands is thus a European conservation priority (Smith 1980, Hillier et al. 1990, Willems 1990, WalliesDeVries et al. 2002). The conservation and/or restoration of these habitats are still highly dependent on active conser-

vation and restoration practices and require robust scientific knowledge on habitat disturbance and grassland resilience.

The resilience of calcareous grasslands after cultivation has already been studied in Europe. Former cultivations might change species composition, decrease species richness, increase soil fertility, change soil structure and thus might affect the direction taken by secondary successions comparatively to adjacent calcareous grassland (Gibson and Brown 1992, Gough and Marrs 1990). Cultivations also introduce ruderal species which have persistent seeds in the soil seed bank (Graham and Hutchings 1988a,b, Dutoit and Alard 1995a, Koncz et al. 2011). While the short-term effects of cultivation on present-day calcareous vegetation have been clearly identified (Austrheim and Olsson 1999, Römermann et al. 2005, Hirst et al. 2005), most of these studies concerned dry grasslands which had been disturbed in the 20th century. Very few studies have investigated the impacts of disturbance such as cultivation in former times (18-19th century) on the present day vegetation (Cornish 1954, Wells et al. 1976, Dutoit and Alard 1995b, Alard et al. 2005, Karlik and Poschlod 2009) and more particularly on the soil seed bank. From a fundamental point of view, it is very important

to know whether these plant communities will be impacted by major human disturbances for centuries as already reported in forest vegetation more than 1600 years after human occupation (Dupouey et al. 2002, Plue et al. 2008). However, opportunities for rigorous experimentation on long-term vegetation dynamics are rare and such studies require access to the history of sites over many centuries.

The aim of the current study was to assess the natural recovery potential of limestone grasslands disturbed (cultivation) and abandoned between 1819 and 1913 (i.e. from 1 to 2 centuries ago) compared to grassland grazed for centuries (control). Both vegetation (above-ground vegetation, seed banks) and soils (physical and chemical properties) were studied. We hypothesize that the impact of former cultivation still result in persistent soil modifications and changes in above-ground vegetation and seed banks even one or two centuries after the abandonment of cultivation.

Material and methods

Study area

The study was carried out in the nature reserve of Grand-Pierre and Vitain valleys located in the Centre of France 10 km from Blois (47°39'36.58"N, 1°17'26.69"E). This reserve covers 296 ha and altitude varies from 90 to 110 meters between the bottom of the valleys and the edges of the plateau. The substrate is uniform and composed of a hard type of limestone, the limestone of Beauce (Arlot and Hesse 1981). The climate is temperate with a mean annual temperature of 12.4°C and a yearly precipitation of 635 mm.

The vegetation of the nature reserve is a mosaic of limestone grasslands: some have been grazed for centuries (old grasslands) and some have developed on fields abandoned between 1819 and 1913 (abandoned fields), as shown by old postcards, aerial photographs, old documents and land-surveys (Arlot and Hesse 1981). Mowing, sheep grazing and shrub-clearing are the conservation management measures that have been applied in the nature reserve since its foundation in 1982 (Maubert 2000).

Study sites

The history of 3 sites—with similar orientation and slope inclination—was reconstructed with the help of documentary evidence such as French land surveys, military survey maps, old postcards and pictures, old country and regional flora, and aerial photographs (1947–2004 with gaps). Cultivation seems to have been abandoned at the three sites at the beginning of the 20th century and grazing ceased in the 1950's (Arlot and Hesse 1981). Consequently, the three sites were totally abandoned from 1950 to 1980. Since September 1981, when the nature reserve was founded, the three sites have been managed for the conservation of species-rich limestone plant communities. The first site has been mowed and the hay removed every September since 1981. The second site has been extensively grazed by an old breed of sheep reintro-

duced for conservation purposes since 1985. The third site was not managed anymore, but was grazed in 1986 and cleared once in 1993 (Maubert 2000).

At each of these 3 sites, both Old Grassland never cultivated (OG1, OG2 or OG3) and Abandoned Fields (AF1, AF2 or AF3) were identified. Abandoned fields were formerly cultivated with cereals. Historically, farmers selected the most fertile areas of the site to cultivate (Arlot and Hesse 1981). In general, those areas were flatter, with deeper soil, or at the bottom of a slope and so held moisture for longer. Thus, they would always have had a slightly different flora to the less suitable areas that were not chosen for cultivation (Smith 1980, Wells et al. 1976). To avoid these biases, the 3 sites were located more than one kilometer apart. They were carefully chosen in different parts representative of the geological substratum, soils and relief of the nature reserve. Then, the difference between the former land-uses (AF vs OG) could not be attributed to different geo-morphological situations but to difference land-use management during the 19th century (Arlot and Hesse 1981). The distance between each pair of AF and OG was less than 100 meters.

Seed bank and vegetation sampling

The persistent soil seed bank was sampled in February 2003, when species with transient seed banks (Types I *sensu*, Thompson and Grime 1979) are not abundant in the soil (Davies and Waite 1998). In order to collect all seeds buried by former ploughing, soil was sampled down to the bedrock at 20 cm depth. Two depth classes were sampled since Bakker et al. (1996) reported a relationship between depth and the age of seeds. At each of the three AF (AF1, AF2, AF3) and three OG (OG1, OG2, OG3) sites, we randomly positioned 10 quadrats of 1 – 1 m². In each quadrat, 3 cylindrical cores of 7.25 cm diameter were taken to a depth of 0-10 cm and to a depth of 10-20 cm. The 3 samples of a same depth were pooled in order to have only 2 soil samples per quadrat. The total sampling volume per m³ and per depth was 1200 cm³. Soil samples were crumbled, spread out on plastic trays and placed at 4°C for a week. The seed bank was cultivated applying a standard procedure recommended by Ter Heerdt et al. (1996). Soil samples were concentrated by washing them with water on one sieve of 200 µm mesh size to reduce bulk and clay. The concentrated soil was spread in trays filled with 2 cm vermiculite topped with medical compresses (100 µm mesh size). The trays were watered frequently from below. To accelerate germination and prevent exogenous species contamination, the trays were placed in a greenhouse and covered with fine gauze material. Emerging seedlings were identified (Muller 1978, Mamarot 2002) and removed or replanted for later identification (Jauzein 1995). After two months, soil samples were carefully turned over in order to facilitate the emergence of new seedlings (Roberts 1981). After five months, sampling was stopped as no new seedling had emerged for several consecutive weeks.

In each of the three AF and three OG sites, 10 vegetation quadrats were placed close to the seed bank quadrats to com-

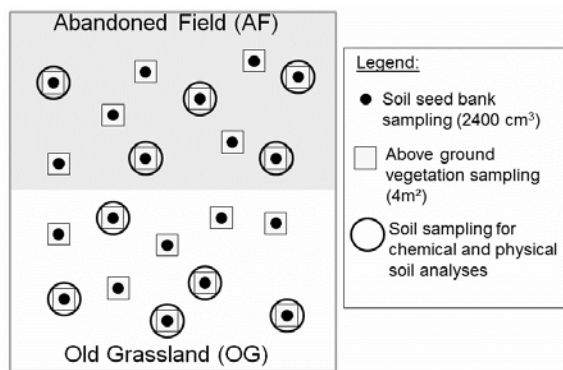


Figure 1. Sampling design used for the three sites. Vegetation quadrats were randomly selected within AF or OG and were positioned away from edges to avoid edge effects. Distance between AF and OG was less than 100 meters.

pare seed bank and above-ground vegetation data (Fig. 1). Vegetation was sampled using the frequency method (Sutherland 1996) in $2 \times 2 \text{ m}^2$ quadrats divided into $25 \times 0.16 \text{ m}^2$ sub-quadrats. Abundance of each plant species was thus expressed in percentage per 4 m^2 using absence-presence of each species within the 25 sub-quadrats. Vegetation was sampled in May 2003 when vegetation development was at its optimum. Plant abundance, percent cover of stone, bare-ground and moss-lichen layer were estimated in each quadrat.

Soil samples

For soil analyses, five soil samples were taken at each of the three AF and three OG sites (total 30 samples). Soil sample positions were randomly chosen among five of the ten previous vegetation quadrats (Fig. 1). The soil samples were collected from 0 to 20 cm depth at each site using a drill. After drying and sieving ($200 \mu\text{m}$), chemical analyses (CaCO_3 , P_2O_5 , CaO , MgO , K_2O , C, N and pH) were carried out by the Laboratory of Soil Analysis of INRA (Arras, France) using the methods illustrated by Baize (2000). CaO , MgO and K_2O were analyzed by spectrophotometry after extraction with ammonium metavanadate. P_2O_5 was quantified using the Olsen method, CaCO_3 was measured by calcimeter, C by Anne method and total N by Kjeldahl method. pH was measured in a slurry with water. The organic matter (OM) of the samples was estimated from the organic carbon content (C) multiplied by 1.72 (Navarro et al. 1993). Fine granulometry analyses (Aubert 1970) were also performed with the Robinson method to quantify percentage content of clay, fine and coarse silt, and fine and coarse sand.

Data analyses

We performed a co-inertia analysis (Dolédéc and Chesnel 1994) in order to study the complex relationships between above-ground vegetation composition and environmental factors. Co-inertia analysis is an adequate way to couple two data matrices (plant species data and environmental variable data) when the number of environmental variables is high.

Only half of the flora matrix ($64 \text{ species} \times 60 \text{ sample quadrats}$) was used in this analysis in order to have soil data for each sample. We first performed a Principal Component Analysis (PCA) on the floristic matrix ($64 \text{ species} \times 29 \text{ samples}$) and a Principal Component Analysis (PCA) on the environmental matrix ($15 \text{ variables} \times 29 \text{ samples}$). The environmental variables included 8 soil chemistry variables (CaCO_3 , P_2O_5 , CaO , MgO , K_2O , C, N, pH) and 7 soil physical variables (clay, fine and coarse silts, fine and coarse sand, percent cover of stone and bare-ground). Analyses were performed on 29 (and not 30) samples because in AF3 abnormal chemical soil results were obtained (most probably due to rabbit holes). Rare species occurring in fewer than 5% of samples were eliminated. The results of the two analyses were associated through a co-inertia analysis (Dolédéc and Chesnel 1994). The statistical significance of co-inertia was tested using a Monte-Carlo 1000-permutation test (Chessel 1997). These analyses were performed with the ade4 package (Chessel et al. 2004) of the R software (R Development Core Team, 2008).

Species richness per quadrat (4 m^2) was calculated for the above-ground vegetation. Mean numbers of species and seeds were calculated for the soil seed bank at 0-10 cm, 10-20 cm and at 0-20 cm for each sample. Data on species richness, seed density, percent cover of mosses and lichens, stone and bare-ground ($n = 10$), as well as soil nutrients and granule sizes ($n = 5$) were analyzed using mixed model ANOVA testing for ancient cultivation effect (AF vs OG). We included (1) Site as a random effect and (2) Ancient cultivation effect as a fixed effect nested within Site. These analyses were performed with JMP Statistical software (version 3.5.1; SAS Institute, Cary, NC).

Sørensen's similarity indices (C_s) were calculated in order to measure similarities between seed banks (0-10, 10-20, 0-20 cm) and between seed banks and above-ground vegetation by pooling data from the three abandoned fields (AF) and the three old grasslands (OG). Then, a protest analysis (PROcrustean randomized TEST) was run. Protest is a randomisation test able to assess the concordance between two sets of variables observed on the same samples (Jackson 1995). This test is also relevant to detect non-linear correlations between matrices.

Results

Soil chemistry and soil physics

The statistical significance of the co-inertia (total inertia 2.84) was high ($p < 0.001$) according to the results of the 1000-permutation Monte-Carlo test. The first two axes of the co-inertia accounted for 79% of the total variation with a contribution of 60% to axis 1 (covariance 1.306) and 19% to axis 2 (covariance 0.735).

The 5 samples of each of the three AF (AF1, AF2, AF3) and three OG (OG1, OG2, OG3) sites were relatively clumped in the factorial plan (Fig. 2a). The projection of plots clearly separated old grassland from abandoned fields

at each site. However, the co-inertia also showed an important site effect with an opposition between sites 1, 2 and 3 depending on the factorial axis (Fig. 2a.). This strong site effect was confirmed by univariate analyses showing significant differences between sites for all soil variables, except for CaCO₃ content and percentage of stone and bare ground (Table 1 in Electronic Appendix, Fig. 2b).

Most soil variables showed significant differences between AF and OG. Only P₂O₅, C/N, Clay, silt and cover of bare ground showed no differences (Table 1 in Electronic Appendix).

Above-ground vegetation composition and richness

Plant community structure and composition are reported in Figure 2c. Among the 6 dominant species of the above-ground vegetation (Table 2 in Electronic Appendix), 4 species showed significant differences in abundance between AF and OG: *Brachypodium pinnatum* (F=19.02, p<0.0001), *Carex caryophylla* (F=17.44, p<0.0001), *Hippocrepis comosa* (F=8.86, p<0.0001) and *Seseli montanum* (F=5.65, p=0.002). The abundance of these species changed depending on the site (mainly sites 1 and 2 vs site 3) and the ancient

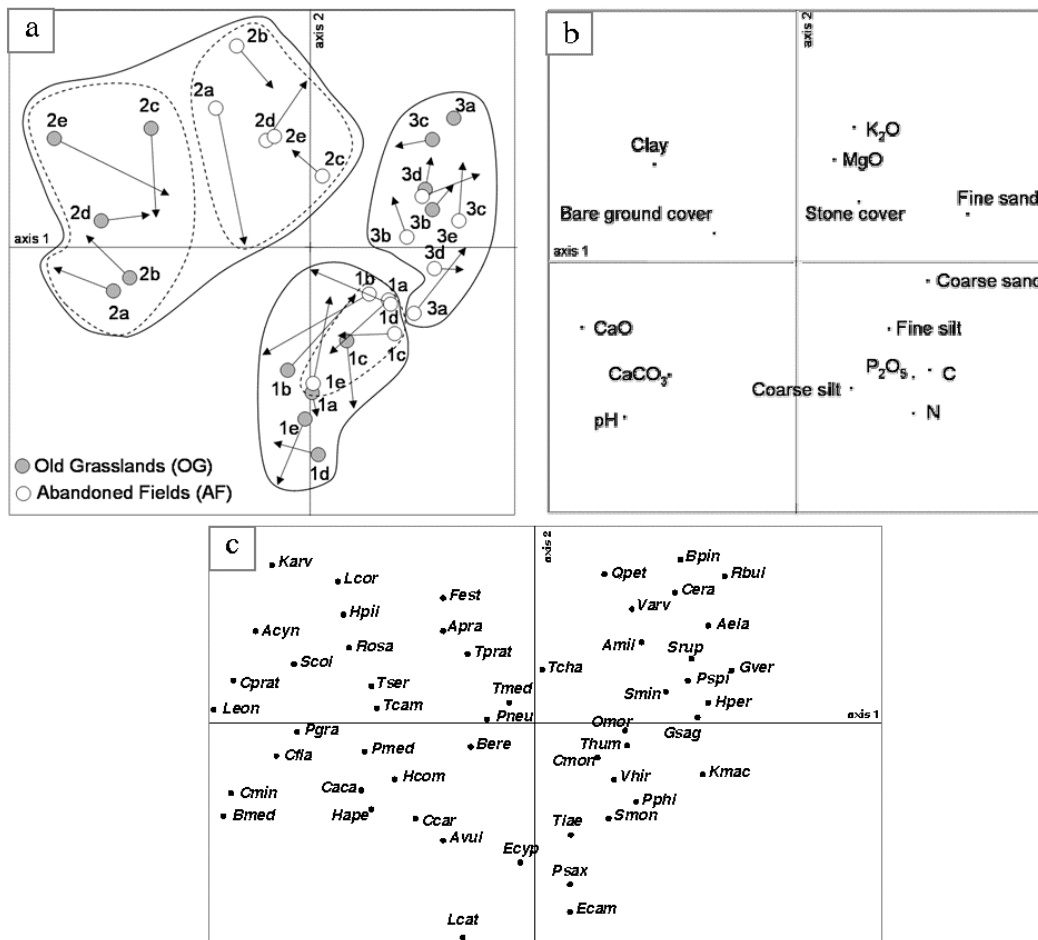


Figure 2. Co-inertia analysis (axes 1-2) of the above-ground vegetation of the nature reserve of Grand-Pierre and Vitain valleys. Matrix: 53 species, 29 quadrats, 15 variables. (a) Site projections: circles correspond to the type of grasslands (grey circle for old grasslands and white circles for abandoned fields) and the site numbers (1, 2 or 3) are shown by their labels. The ordination of sites is based on environmental data (arrow bases) and plant species data (arrow heads). Short arrows indicate that the plant and environmental data occupy similar positions in the ordination space. (b) Variables (see text for the significance of variables). (c) species projection. Amil: *Achillea millefolium*, Acyn: *Asperula cynanchica*, Aela: *Arrhenatherum elatius*, Apra: *Avenula pratensis*, Avul: *Anthyllis vulneraria*, Bere: *Bromus erectus*, Bmed: *Briza media*, Bpin: *Brachypodium pinnatum*, Caca: *Cirsium acaule*, Ccar: *Carex caryophylla*, Cera: *Cerastium sp.*, Cfla: *Carex flacca*, Cmin: *Coronilla minima*, Cmon: *Crataegus monogyna*, Cprat: *Centaurea pratensis*, Ecam: *Eryngium campestre*, Ecyp: *Euphorbia cyparissias*, Karv: *Knautia arvensis*, Fest: *Festuca sp.*, Kmac: *Koeleria macranta*, Gver: *Galium verum*, Gsag: *Genista sagittalis*, Hape: *Helianthemum apenninum*, Hcom: *Hippocrepis comosa*, Hper: *Hypericum perforatum*, Hpil: *Hieracium pilosella*, Lcat: *Linum catharticum*, Lcor: *Lotus corniculatus*, Leon: *Leontodon sp.*, Omor: *Orchis morio*, Pgra: *Prunella grandiflora*, Pmed: *Potentilla neumannia*, Pneu: *Potentilla neumannia*, Pphi: *Phleum phleoides*, Psax: *Pimpinella saxifraga*, Pspi: *Prunus spinosa*, Qpet: *Quercus petraea*, Rbul: *ranunculus bulbosa*, Rosa: *Rosa sp.*, Scol: *Scabiosa columbaria*, Smin: *Sanguisorba minor*, Smon: *Seseli montanum*, Srup: *Sedum rupestre*, Tcam: *Trifolium campestre*, Tcha: *Teucrium chamaedrys*, Thum: *Thesium humifusum*, Tlae: *Taraxacum laevigatum*, Tmed: *Trifolium medium*, Tprat: *Trifolium pratense*, Tser: *Thymus serpyllum*, Varv: *Veronica arvensis*, Vhir: *Viola hirta*.

cultivation effect (OG or AF). For example, *Carex caryophylla* was twice as abundant in OG1 and OG2 (respectively 56.4% and 70%) as in AF1 and AF2 (25.6% and 32.8%). Abundance of *Brachypodium pinnatum* was also 4 times higher in AF1 and AF2 (respectively 68.8% and 76.4%) than in OG1 and OG2 (13.2% and 18.8%).

OG had significantly higher species richness per 4 m² ($F = 15.32, p < 0.001$) and higher cover of moss-lichen layer ($F = 5.73, p < 0.01$) than adjacent AF (Table 3 in Electronic Appendix). In addition, AF sites were characterized by a highest density of ligneous individuals (mainly *Prunus spinosa* and *Crataegus monogyna*) with a total of 28 individuals in all AF and only 6 in all OG.

Seed bank

2217 seedlings emerged in the investigation of the seed bank out from 120 soil samples in the 6 sites at both depths. 1836 seeds emerged from the upper layer (0-10 cm) and only 381 from the deeper layer (10-20 cm). We identified 43 species in the seed bank. Altogether, 15 species were present only in the soil seed bank, 42 only in the above-ground vegetation and 28 common to both seed bank and vegetation.

No significant differences in the density of seeds were found between AF and OG (Table 3 in Electronic Appendix). Seed banks were dominated by *Linum catharticum* (which is a species with a high longevity (100 yr) according to Thompson et al. 1997), then by *Hypericum perforatum* (longevity > 40 yr) and *Potentilla neumanianna* (longevity > 30 yr) making up more than 73% of all emerged seedlings at the 0-10 cm depth and 56% at the 10-20 cm depth. Most of the frequent species of the above-ground vegetation were poorly represented in soil seed banks except *Carex caryophylla* (Table 2 in Electronic Appendix). There were 7 species only present in the AF seed banks, 8 in the OG seed banks and 26 present in both. Species richness per sample (1200 cm³) was higher in OG than in AF (Table 3 in Electronic Appendix). Some arable weed species and ruderal species were only found in the soil seed bank but not in the above-ground vegetation (*Anagallis arvensis*, *Erodium cicutarium*, *Solanum nigrum*, *Sonchus asper*, *Sonchus oleraceus*, etc.). Among these species, we found *Althaea hirsuta*, a very rare arable weed in France, in the soil seed bank of the abandoned field AF3.

Comparing the Sørensen's similarity indices of seed bank at two sampling depths within each site, we found that the seed banks were more similar in OG ($C_s = 0.640 \pm 0.079, p < 0.05$) than in AF ($C_s = 0.480 \pm 0.019, p < 0.05$). Seed banks and above-ground vegetation composition were not significantly similar; however, the seed bank of OG tended to be more similar to the above-ground vegetation than those of AF. Protest analysis showed that species frequency in the seed bank was slightly more similar to above-ground vegetation in OG (0-10 cm: $F = 0.7914, p < 0.05$; 10-20 cm: $F = 0.731, p < 0.001$) than in AF (0-10 cm: $F = 0.786, p < 0.01$; 10-20 cm: $F = 0.6719, p < 0.01$).

Discussion

The aim of this study was to investigate long-term resilience of limestone grassland communities after cultivation. This study shows that soil properties and plant communities (seed bank and above-ground vegetation) were still impacted one century after abandonment of their cultivation. Similar results in other ecosystems—forest ecosystems (France)—have also shown that ancient human land-uses (1600 year old Gallo-Roman occupation) leave a persistent trace on forest soil, above-ground vegetation and seed bank (Dupouey et al. 2002, Plue et al. 2008). The resilience of calcareous grassland is very long or may not be reached again after long-lasting disturbance. To our knowledge, no other study analyzed the long-term effects (centuries) of cultivation on present-day on seed banks' composition and richness in limestone grasslands.

Effects of ancient cultivation on soil properties

In this study, former cultivations led to geochemical alterations compared to old grasslands which were never cultivated. Most soil variables (pH, CaCO₃, CaO, MgO, C, coarse sand, etc.) were significantly different between old grasslands and abandoned fields. Stone cover was lower on abandoned fields. During the period of cultivation, stones were probably removed by hand to facilitate ploughing (Dutoit et al. 2003a). Formerly cultivated soils (AF) had also significantly lower pH, CaCO₃ and CaO content (sites 1 and 2 particularly) because former agricultural practices left soil surface bare in winter which increased the leaching of carbonate and calcium (Dutoit et al. 2003a). Some nutrients, such as K₂O, were higher in AF than in OG. Farming practices in the 19th century (ploughing, stubble grazing, manuring) increased the soil nutrient status of AF. These practices were less intensive than modern farming practices: fertilising was limited to organic manure and ploughing to less than 20 cm deep. Moreover, time since abandonment was long (up to 185 years), leaving time for amendments to leach out, for succession to occur and for the arable weed community to change towards the calcareous grassland community (Wells et al. 1976, Gibson and Brown 1991, Karlik and Poschlod 2009). The effects of former cultivations on the current vegetation composition are thus likely to be less severe than those of modern cultivation (Graham and Hutchings 1988a,b, Buisson and Dutoit 2004, Römermann et al. 2005, Buisson et al. 2006).

Effects of ancient cultivation on above-ground vegetation

Species richness (from above-ground vegetation and upper layer of the seed bank) was still lower in AF one century after the abandonment of cultivation. Similar results have been obtained for the calcareous grasslands of the United Kingdom (Cornish 1954, Wells et al. 1976). Karlik and Poschlod (2009) found no difference in the species richness of ancient (150 years old grassland) and recent grasslands (50

to 60 years old), although their species composition differed. In this study, we did not find in the above-ground vegetation species or markers of previous past cultivations. After abandonment of crops in the nature reserve of Grand-Pierre and Vitain valleys, sheep grazing was re-introduced immediately. It is a common and successful management tool which enhances seed dispersal from dry grasslands to abandoned fields (Gibson and Brown 1992, Fischer et al. 1996, Mouissie et al. 2005) and contributes to homogenize sites (Gibson et al. 1987). Lastly, the absence of field species markers of AF and OG may be explained by a low distance between them (less than 100 meters) and a continuous seed input from OG to AF was maintained.

Our results also suggested that the vegetation of OG was more open and less dynamic than on AF. The first axis of the co-inertia might reflect the natural successional dynamic of these limestone grasslands, with younger successional stages on the left and later successional stage on the right of the first axis (for sites 1 and 2). We found that *Brachypodium pinnatum* was more abundant in AF than in OG. Experiments in *Bromus* community demonstrated the competitive superiority of *Brachypodium pinnatum* ssp. *rupestre* over *Bromus erectus* (Corcket et al. 2003, Liancourt et al. 2005) suggesting that *B. pinnatum* abundance increases along grassland succession. *B. pinnatum* is well known to produce a large amount of recalcitrant litter (Bobbink and Willems 1987) and forms dense mats which contribute to reducing species richness (Bobbink and Willems 1987). The lower cover of moss-lichen layer on AF could be explained by a higher competition by herbaceous species. Cryptogamic species are generally regarded as being poor competitors for resources such as light and moisture (Eldridge et al. 2000). In addition, AF sites were characterized by the highest density of ligneous individuals (mainly *Prunus spinosa* and *Crataegus monogyna*) with 28 individuals in all AF and only 6 in all OG.

Effects of ancient cultivation on soil seed bank

After 185 years of abandonment, changes in species richness were still recorded between OG and AF in both layers of the soil seed bank. Dölle and Schmidt (2009) showed that seed banks of pastures created on former arable fields have been found to be species poor.

No difference in seed density was found between AF and OG, conversely to other studies on shorter time of abandonment after cultivation (Graham and Hutchings 1988a,b, Austrheim and Olsson 1999, Römermann et al. 2005). Contrary to our expectation, former ploughing in AF did not lead to a significant and strong increase in seed density in the soil. The absence of difference in seed density may also be explained by the method used to investigate seed density (only germinated seeds were counted) and by the decrease in seed viability with time (Bossuyt et al. 2006, Bossuyt and Honnay 2008).

An interesting result of this study was the presence of arable weed species and ruderal species in the soil seed bank of

AF but not in the above-ground vegetation. These species demonstrated that markers of former cultivation were still present in the below ground vegetation. Former ploughing might have buried seeds of arable weed species with long-term persistence. *Althaea hirsuta*, found in the soil seed bank of the abandoned field of site 3, is a very rare arable weed characteristic of cereal cultivation on limestone soils for this region (Botté 1993). This species has not been inventoried for a long time in the nature reserve (Maubert and Dutoit 1995) nor in the region according to Botté (1993) and to other historical documents of the 19th-20th centuries. *Althaea hirsuta* (Malvaceae) has a long-term persistent seed bank (Thompson et al. 1997) and a similar Malvaceae species, *Malva sylvestris*, has seeds living up to 90 years in the soil (Odum 1965). There is a growing interest in the restoration of arable endangered species due to the impact of modern agriculture. This study shows that soil disturbances will not bring many arable weed seeds to the soil surface in this nature reserve. Except for *Althaea hirsuta*, most of the calcareous arable weeds seem to have transient or short-term persistent seed banks (Thompson et al. 1997, Dutoit et al. 2003b). The seed reservoirs of arable weeds thus become depleted with time (Milberg and Hansson 1994) in only several decades.

Relationship of established vegetation and soil seed bank

There were differences between the composition and the structure of the above-ground vegetation and of the seed bank, as observed in other studies (Dutoit and Alard 1995b, Dutoit et al. 2003a, Römermann et al. 2005). In a 36-year-old permanent plot study, Dölle and Schmidt (2009) shown that the ability of a plant community to regenerate from the soil seed bank dramatically decreases with increasing time since abandonment and with decreasing disturbance intensity. Many species of the above-ground vegetation were, if not absent, only scarce in the seed bank. Species with very low frequencies did not emerge at all from the seed bank, similarly to the findings of Milberg and Hansson (1994). The seed bank composition was not related to the rarity or abundance in the above-ground vegetation, a phenomenon observed in other seed bank studies in calcareous grasslands (Thompson and Grime 1979, Dutoit and Alard 1995b). We found that similarity between upper and deeper layers of the soil seed bank, and between soil seed bank and above ground vegetation was slightly higher for OG than for AF, suggesting that former cultivation potentially still impacts plant communities' structure and composition in space and time.

Importance of present vs. former management regimes

Despite a significant effect of ancient cultivation on soil and vegetation (above-ground and seed bank), we found an important site effect. In their study, Karlik and Poschold (2009) found that history (former land use, age of habitats) affected the vegetation pattern more than environmental factors (geology) in calcareous grassland communities. Our site effect is likely related to the present conservation measures

in the reserve. We are aware that our experimental design was not well-suited to test the effect of present management or to test the relative importance of present vs former management effect. Nevertheless we can put forward the hypothesis that the ancient cultivation effect (OG vs AF) might be deviated by the current management regime, making generalization quite difficult. Jacquemyn et al. (2003, 2011) found that the impact of the current management regime in limestone grassland (mowing, grazing, no management) affects more strongly the structure and composition of the above-ground vegetation and soil seedbanks than former cultivation one century after abandonment. Gustavsson et al. (2007) also investigate the relative importance of historical grassland management (about 150 years ago) and current land use for grassland plant diversity in southwest Swedish grasslands. They showed that diversity patterns reflected the spatial variation of historical grassland management more than that of current land use, both in currently grazed and abandoned grasslands. They also found that the sequential change of land use from the 18th century to 1961 explained more the variation in plant diversity patterns than the current land use.

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Appendix

Table 1. Comparison of soil parameters between old grasslands (OG) and abandoned fields (AF) for the three studied sites.

Table 2. Dominant species of the soil seed banks and the above-ground vegetation (n=10) in the abandoned fields and the old grasslands for the three studied sites.

Table 3. Species-richness of above-ground vegetation, density of germinated seeds and species in the soil seed bank of old grasslands (OG) and abandoned fields (AF).

The file may be downloaded from the web site of the publisher at www.akademai.com.

Table 1. Comparison of soil parameters between old grasslands (OG) and abandoned fields (AF) for the three studied sites. Means and standard deviation are given (n = 5 or 10). Sites and ancient cultivation effects were tested using a mixed-model analysis.

	old grasslands (OG)			abandoned fields (AF)			ancient cultivation effect	Site effect
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3		
pH	7.26 ± 0.17	7.34 ± 0.16	6.54 ± 0.14	7.20 ± 0.15	6.54 ± 0.12	6.52 ± 0.15	F = 62.43 p < 0.001	F = 27.23 p < 0.001
CaCO₃ (g/kg)	2.2 ± 0.8	2.2 ± 1.1	1 ± 0.0	1.2 ± 0.4	1 ± 0.0	1.2 ± 0.4	F = 5.22 p < 0.01	F = 2.19 p = 0.134
CaO (g/kg)	9.27 ± 0.49	10.68 ± 0.42	6.71 ± 0.44	7.92 ± 0.67	8.70 ± 0.32	7.57 ± 1.15	F = 20.86 p < 0.001	F = 77.96 p < 0.001
MgO (g/kg)	0.37 ± 0.03	0.37 ± 0.04	0.45 ± 0.07	0.37 ± 0.05	0.49 ± 0.03	0.43 ± 0.13	F = 7.77 p < 0.001	F = 4.05 p < 0.05
K₂O (g/kg)	0.19 ± 0.02	0.24 ± 0.03	0.28 ± 0.07	0.17 ± 0.03	0.32 ± 0.05	0.43 ± 0.25	F = 3.03 p < 0.05	F = 19.56 p < 0.001
P₂O₅ (mg/kg)	13.2 ± 2.9	6.2 ± 0.8	10.2 ± 2.1	11.6 ± 2.7	7.8 ± 3.1	16.6 ± 10.0	F = 1.16 p = 0.34	F = 12.69 p < 0.001
N (g/kg)	5.96 ± 0.38	3.47 ± 0.29	4.75 ± 0.45	4.81 ± 0.44	3.39 ± 0.2	6.07 ± 2.33	F = 7.46 p < 0.01	F = 65.90 p < 0.001
C (g/kg)	65.7 ± 3.3	46.3 ± 4.9	59.6 ± 6.4	55.5 ± 3.5	46.9 ± 4.0	75.7 ± 29.4	F = 3.58 p < 0.05	F = 25.26 p < 0.001
O.M.(g/kg)	113.0 ± 5.7	79.6 ± 8.4	102.5 ± 11.1	95.4 ± 6.0	80.7 ± 6.8	130.2 ± 50.5	F = 3.59 p < 0.05	F = 25.23 p < 0.001
C: N ratio	11.0 ± 0.2	13.3 ± 0.3	12.5 ± 0.5	11.6 ± 0.5	13.8 ± 0.6	12.5 ± 0.3	F = 2.36 p = 0.097	F = 67.62 p < 0.001
Clay (%)	42.7 ± 1.6	56.6 ± 4.7	45.3 ± 4.0	39.2 ± 1.7	51.5 ± 7.5	40.2 ± 5.1	F = 2.20 p = 0.115	F = 27.47 p < 0.001
Fine silt (%)	24.2 ± 1.7	18.0 ± 3.0	22.4 ± 3.0	25.2 ± 1.2	23.1 ± 6.8	24.6 ± 2.1	F = 1.97 p = 0.146	F = 3.68 p < 0.05
Coarse silt (%)	25.2 ± 1.5	19.4 ± 3.7	19.6 ± 4.7	23.1 ± 1.5	19.5 ± 1.3	25.2 ± 3.1	F = 2.32 p = 0.101	F = 7.36 p < 0.01
Fine sand (%)	3.48 ± 0.19	2.98 ± 0.32	4.63 ± 0.84	4.50 ± 0.46	3.45 ± 0.38	4.63 ± 0.90	F = 3.52 p < 0.05	F = 17.60 p < 0.001
Coarse sand (%)	4.42 ± 0.87	3.01 ± 0.37	8.10 ± 1.82	8.03 ± 1.2	2.46 ± 0.35	5.14 ± 1.11	F = 15.69 p < 0.001	F = 39.32 p < 0.001
Stone cover (%)	0.4 ± 0.9	0.4 ± 0.9	4.0 ± 4.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	F = 3.64 p < 0.01	F = 3.00 p = 0.069
Bare ground (%)	3.6 ± 4.2	8.4 ± 12.1	2.0 ± 2.7	0.0 ± 0.0	3.4 ± 1.7	0.0 ± 0.0	F = 1.14 p = 0.351	F = 2.22 p = 0.131

Table 2. Dominant species of the soil seed banks and the above-ground vegetation (n=10) in the abandoned fields and the old grasslands for the three studied sites. The 6 most dominant above-ground species are reported in the table and are expressed in percentage (%) of species abundance in the 4 m² x 10 quadrats. For the seed bank, the 9 most abundant seed species are expressed in percentage of seed density in the sampled soil (1200 cm³ x 10 cores).

		SOIL SEED BANK (SEED %)						ABOVE-GROUND VEGETATION (ABUNDANCE %)					
		old grasslands (OG)			abandoned fields (AF)			old grasslands (OG)			abandoned fields (AF)		
Species		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Dominant species of the seed bank	<i>Carex caryophylla</i>	2.7	28.7	0	6.4	36.8	2.6	56	70	3	26	33	89
	<i>Galium verum</i>	0.3	0	6.5	0	0.5	0.4	25	0.5	48	2	0.5	39
	<i>Hippocrepis comosa</i>	3.4	2.4	7.6	5.3	1.1	0	90	97	60	96	84	8
	<i>Hypericum perforatum</i>	6.1	0.7	16.6	13.1	1.6	34	4	0.5	23	5	1	46
	<i>Linum catharticum</i>	62.3	49.5	25.4	52.8	34.6	27	50	27	5	38	16	10
	<i>Potentilla neumannia</i>	9.3	4.5	14.3	10.9	4.4	15	48	65	65	41	47	37
	<i>Sanguisorba minor</i>	0.3	0.7	5	1.1	0	0.4	14	6	60	14	7	3
	<i>Teucrium chamaedrys</i>	2.9	0.7	7.1	1.3	3.3	4.9	91	72	85	48	80	100
	<i>Trifolium campestre</i>	1.9	0.3	0.8	0.5	3.3	1.3	0	2	0	0	0	0
Dominant species of the above-ground vegetation	<i>Brachypodium pinnatum</i>	0	0	0	0	0	0	13	19	74	69	76	73
	<i>Bromus erectus</i>	0	5.2	0.8	0.5	0	0	96	100	100	100	100	100
	<i>Carex caryophylla</i>	2.7	28.7	0	6.4	36.8	2.6	56	70	3	26	33	89
	<i>Festuca sp.</i>	0	0	0.6	0.8	1.1	1.1	99	100	100	95	100	89
	<i>Hippocrepis comosa</i>	3.4	2.4	7.6	5.3	1.1	0	90	97	60	96	84	8
	<i>Seseli montanum</i>	0.8	0	2.3	0.5	0.5	0	96	87	97	91	70	69

Table 3. Species-richness of above-ground vegetation, density of germinated seeds and species in the soil seed bank of old grasslands (OG) and abandoned fields (AF). The total and mean numbers \pm standard deviations of seed density and richness are shown separately for each sampled site. Sites and ancient cultivation effects were tested using a mixed-model analysis.

	old grasslands (OG)			abandoned fields (AF)			ancient cultivation effect	Site effect		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3				
<i>Vegetation</i>										
Total number of species in the vegetation (40 m ²)	44	47	37	38	42	47				
Species richness (per 4m ²)	23.6 \pm 2.3	29.3 \pm 2.4	24.6 \pm 1.3	20.7 \pm 1.8	23.1 \pm 2.9	23.7 \pm 2.7	F = 15.32	<i>p</i> < 0.001	F = 15.82	<i>p</i> < 0.001
Bryo-lichens cover (%)	66.5 \pm 17.7	75.0 \pm 17.8	72.0 \pm 14.0	60.0 \pm 6.7	57.0 \pm 18.9	53.0 \pm 6.8	F = 5.72	<i>p</i> < 0.01	F = 0.039	<i>p</i> = 0.727
<i>Soil Seed bank</i>										
Total number of species in the sampled seed bank	18	20	25	18	20	20				
Species of above-ground vegetation in the soil seed	13%	8%	16%	14%	10%	12%				
Mean number of species at 0-10cm (per m ²)	1468 \pm 387	1262 \pm 464	2190 \pm 413	1340 \pm 516	902 \pm 207	1649 \pm 387	F = 4.70	<i>p</i> < 0.01	F = 23.04	<i>p</i> < 0.001
Mean number of species at 10-20 cm (per m ²)	644 \pm 413	438 \pm 284	1314 \pm 413	593 \pm 335	722 \pm 593	619 \pm 387	F = 5.93	<i>p</i> < 0.01	F = 5.69	<i>p</i> < 0.01
Mean number of species at 0-20 cm (per m ²)	799 \pm 284	1159 \pm 181	1108 \pm 335	928 \pm 181	1005 \pm 335	799 \pm 232	F = 7.68	<i>p</i> < 0.001	F = 23.26	<i>p</i> < 0.001
Total number of germinated seeds	377	289	523	376	182	470				
Mean number of seeds at 0-10 cm (per m ²)	8602 \pm 6671	6696 \pm 4379	10894 \pm 3220	7855 \pm 8164	2679 \pm 2087	10559 \pm 5409	F = 0.95	<i>p</i> = 0.422	F = 6.26	<i>p</i> < 0.01
Mean number of seeds at 10-20 cm (per m ²)	1108 \pm 593	747 \pm 593	2576 \pm 1468	1829 \pm 1108	2009 \pm 4044	1546 \pm 1571	F = 1.28	<i>p</i> = 0.259	F = 0.72	<i>p</i> = 0.493
Mean number of seeds at 0-20cm (per m ²)	4765 \pm 3323	3606 \pm 2447	6696 \pm 1829	4765 \pm 3889	2318 \pm 2035	6053 \pm 3323	F = 0.47	<i>p</i> = 0.704	F = 6.76	<i>p</i> < 0.01