The importance of plant-soil interactions and plant life history traits for the population dynamics of Jacobaea vulgaris during old-field succession

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The importance of plant–soil interactions, soil nutrients, and plant life history traits for the temporal dynamics of *Jacobaea vulgaris* in a chronosequence of old-fields

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We examined to what extent temporal dynamics of *Jacobaea vulgaris* cover in old-fields were related to plant–soil feedback, soil nutrients, seed availability and performance, and seedling establishment. Long-term measurements at an experimental field and in ten old-fields representing a chronosequence following land abandonment revealed a remarkably similar hump-shaped temporal pattern of *J. vulgaris* cover, which peaked at about five years after abandonment. In a plant–soil feedback study, *J. vulgaris* biomass of plants grown in soil from all chronosequence fields was lower than in sterilized control soil. However, biomass of *J. vulgaris* in the feedback study was lower when grown in soil collected from fields with a high density of *J. vulgaris* plants than in soil from fields with a low density of *J. vulgaris*. When plants were grown again in the conditioned soil, a strong negative plant–soil feedback response was observed for soils from all fields. These results indicate that soils from all stages of the chronosequence can develop a strong negative soil feedback to *J. vulgaris*, and that there is a positive relationship between *J. vulgaris* density and the subsequent level of control by the soil community. In a common-garden experiment with turfs collected from the chronosequence fields in which *J. vulgaris* was seeded, seedling establishment was significantly lower in turfs from older than from young fields. In a seed bank study the number of emerging seedlings declined with time since abandonment of the field. In conclusion, negative plant–soil feedback is an important factor explaining the hump-shaped population development of *J. vulgaris*. However, it is not operating alone, as propagule availability and characteristics, and competition may also be important. Thus, in order to explain its contribution to plant population dynamics, the role of biotic plant–soil interactions, soil nutrients and life history characteristics along successional gradients should be considered from a community perspective.

Why and how the composition of plant communities changes over time is a long-standing issue in ecology. Old-field succession has long been used to understand the mechanisms that determine long-term plant population dynamics (Connel and Slatyer 1977). Many early-successional plant species enter and leave successional sequences according to a boom–bust pattern (Olff and Bakker 1991, Bezemer et al. 2006, Meiners et al. 2009), but there is little coherent understanding of what causes these typical temporal processes (Bardgett et al. 2005). The focus of succession studies has been on detecting how changes in abiotic conditions, such as soil nutrients, and competition with later-successional species influence plant community composition (Tilman and Wedin 1991, Huston 1994, Olff et al. 1994). Other studies have considered plant dispersal and recruitment dynamics, resource availability, and control by natural enemies (Huston and Smith 1987, Olff and Bakker 1991, Brown and Gange 1992, Kardol et al. 2006). However, relatively few studies have taken a more integral approach considering a number of these factors at the same time as drivers of plant population dynamics throughout succession.

Soil biotic interactions are now known to play an important role in influencing the performance and population dynamics of plants (Reynolds et al. 2003, Kardol et al. 2006). For example, pathogenic soil bacteria and fungi, plant-feeding nematodes, and mycorrhizal fungi can have strong effects on the performance of individual plants and on plant community composition (van der Heijden et al. 1998, De Deyn et al. 2003, Kardol et al. 2007). Even when plants are growing in mixed plant communities, they culture species-specific soil communities which include species that can negatively affect the plant itself (Bezemer et al. 2010). When these cultured soil communities have a negative effect on subsequent plant performance of conspecifics, this is called negative plant–soil feedback (Bever et al. 1997). Soil-borne enemies are capable of affecting the long-term population dynamics of plant species (Augspurger 1983, van der Putten et al. 1993, Bever 1994), however, relatively little is known about how strongly these plant–soil interactions may influence plant performance and population dynamics in the field, when also other processes take place.

While the performance of a plant can be greatly influenced by the abiotic and biotic soil environment, the population

size of a plant species is also determined by its seed production and dispersal, and subsequent germination and establishment rates (Chapin et al. 1994, Anderson 2007). Many of these plant life history traits are context dependent, and are, for example, related to the density of the plant species, abiotic conditions of the environment, and biotic interactions with herbivores, pathogens, symbionts and decomposer organisms (Wardle et al. 2004). It is not yet known whether changes in plant life history traits and the occurrence of plant–soil interactions are both important for the population dynamics during old-field succession or that one is more important than the other, and whether this may depend on the successional stage of the field.

Here, we examine how plant–soil biota interactions, plant life history traits, and characteristics of the habitat (soil nutrients) change in a chronosequence and whether these processes relate to the abundance of the early successional species *Jacobaea vulgaris* (syn. *Senecio jacobaea*) in this chronosequence. We studied the population dynamics of this species in a long-term experimental field site and in a chronosequence of ten former arable fields that are converted to species-rich grasslands. *Jacobaea vulgaris* is a plant species that is typical for restored grasslands on relatively dry, sandy soils (van der Meijden and van der Waals-Kooi 1979, Crawley and Nachapong 1985, Bezemer et al. 2006). The plant can become highly abundant during the first years of succession, but then abundance declines (Bezemer et al. 2006). The chronosequence enabled us, within a single field season, to collect soil samples and determine plant characteristics in fields that represent different stages of old-field succession, whereas the data collected annually at a long-term experimental field site enabled us to verify whether the chronosequence would be appropriate for replacing time by space.

Specifically, we examined whether the population decline of *J. vulgaris* in the field coincides with the development of a negative plant–soil feedback, caused by the net build up of a soil pathogenic effect; and whether the population decline of *J. vulgaris* during old field succession is related to changes in plant life history traits or soil nutrient status. We performed a greenhouse plant–soil feedback experiment with soil collected from the old-fields to determine how soil community composition influences *J. vulgaris* performance. In the chronosequence fields we measured *J. vulgaris* characteristics, such as germination, and seed availability, and soil nutrient status. In a common garden experiment we measured seedling establishment in turfs that were collected in the chronosequence fields. We calculated effect sizes of the measured variables to determine their relation to *J. vulgaris* cover and density in the fields. We will discuss the contributions of plant–soil feedback, plant life history trait variation and soil nutrient status to the abundance of *J. vulgaris* in the chronosequence fields.

Material and methods

Jacobaea vulgaris ssp. *vulgaris* (synonym *Senecio jacobaea*) is a monocarpic perennial weed (Asteraceae) that spends its first year as a rosette. Flowering may take place in the second year, but is often delayed following herbivory (van der Meijden

and van der Waals-Kooi 1979). *Jacobaea vulgaris* is an early successional plant species native to the Netherlands and Europe, but invasive in other continents. In the Netherlands and in many other countries, *J. vulgaris* is considered a problem weed in abandoned arable fields that are used for nature restoration (Bezemer et al. 2006). This is mainly due to its dominance and because it contains pyrrolizidine alkaloids that are toxic for livestock (Cameron 1935).

J. vulgaris population dynamics during old-field succession

In a long-term experimental field experiment, we determined the temporal pattern of *J. vulgaris* abundance in five naturally colonized experimental plots of 10×10 m each that had been monitored for 13 years, from 1996 until 2008. These plots were installed in 1996 as part of a biodiversity experiment (van der Putten et al. 2000). All plots were colonized by *J. vulgaris* in 1997. *Jacobaea vulgaris* cover has been recorded each year at peak standing biomass (Bezemer et al. 2006, van de Voorde et al. 2010). The experimental site is situated in the area of field 12 (Table 1).

Chronosequence fields

We selected ten fields where agricultural practices were ceased between two and 25 years ago (Table 1, Table A1, Kardol et al. 2005). All fields of this chronosequence were at least 1 ha in size, extensively grazed and previously cultivated according to crop rotation schemes. The fields are located on the same parent soil: sandy to sandy loam glacial deposits in the central part of the Netherlands (Veluwe). In July 2007, at each field an imaginary W-shaped transect was laid out in a plot of 50×150 m. All soil and vegetation samples were taken along this transect. Vegetation recordings were taken at the five outer-ends of the W-shape. Plant community composition and estimated percentage cover of each species, including *J. vulgaris*, were recorded on a continuous scale by eye in the five 1×1 m quadrats in each field, for a total of 50 observations (10 fields × 5 quadrats per field). Soil samples were taken at ten sampling points at the W-shape in each field, for a total of 100 samples (10 fields × 10 samples per fields).

J. vulgaris plant size and abundance in the chronosequence fields

In order to determine how life history traits of *J. vulgaris* change during succession, in each field, at the ten positions along the transect that were also used for soil collection, the nearest flowering *J. vulgaris* plant was identified. Plant height of these ten plants was recorded. Plants, including rhizosphere soil, were then excavated, stored individually in plastic bags and brought to the laboratory, where aboveground plant parts and roots were separated. Aboveground plant material was oven-dried (70°C for five days) and weighed. In a 3×3 m area located in the middle of each chronosequence field, the number of *J. vulgaris* plants was recorded and the reproductive height of all flowering plants and the diameter of all rosettes were measured.

Table 1. Overview of year of abandonment and soil nutrient content in the chronosequence fields. Mean ($\pm$SE) nutrient content and p-values of ANOVA testing differences between fields are given. Within columns, means followed by the same letter are not significantly different ($p < 0.05$) based on a Tukey HSD test. The outcome and direction of a linear mixed model (REML) with time since abandonment (age) as a continuous factor and field identity as a random factor (DF = 8) are also presented.

Field code	Year of abandonment	P (mg kg^{-1})	% org. C	C:N ratio	Min-N (mg kg^{-1})	pH H$_2$O
2A	2005	86 $\pm$ 2 cd	4.5 $\pm$ 0.2 ab	21.7 $\pm$ 0.5 de	6.5 $\pm$ 0.2 a	6.2 $\pm$ 0.02 d
2B	2005	83 $\pm$ 1 c	5.7 $\pm$ 0.1 c	20.9 $\pm$ 0.3 d	10.3 $\pm$ 0.7 ab	6.0 $\pm$ 0.06 cd
5A	2002	124 $\pm$ 7 e	4.6 $\pm$ 0.2 b	22.8 $\pm$ 0.2 e	7.4 $\pm$ 0.9 a	5.3 $\pm$ 0.02 a
5B	2002	121 $\pm$ 13 de	4.2 $\pm$ 0.3 ab	22.9 $\pm$ 0.6 e	10.7 $\pm$ 1.3 ab	5.4 $\pm$ 0.15 b
12	1995	90 $\pm$ 3 cde	4.2 $\pm$ 0.3 ab	16.8 $\pm$ 0.1 bc	10.6 $\pm$ 0.8 ab	6.1 $\pm$ 0.07 d
13	1994	58 $\pm$ 4 b	3.6 $\pm$ 0.1 a	21.5 $\pm$ 0.2 de	6.2 $\pm$ 0.3 a	5.4 $\pm$ 0.06 a
17	1990	85 $\pm$ 10 c	3.9 $\pm$ 0.2 ab	15.7 $\pm$ 0.3 ab	15.3 $\pm$ 3.7 b	5.4 $\pm$ 0.12 a
19	1988	84 $\pm$ 4 c	4.4 $\pm$ 0.1 ab	14.7 $\pm$ 0.2 a	9.3 $\pm$ 1.3 ab	5.1 $\pm$ 0.03 a
22	1985	85 $\pm$ 5 c	4.7 $\pm$ 0.2 bc	18.2 $\pm$ 0.4 c	11.4 $\pm$ 0.1 ab	5.3 $\pm$ 0.01 a
25	1982	27 $\pm$ 2 a	4.8 $\pm$ 0.1 bc	20.9 $\pm$ 0.5 d	12.2 $\pm$ 2.0 ab	5.8 $\pm$ 0.02 bc
p (fields)		<0.001	<0.001	<0.001	0.02	<0.001
p (REML ~ age)		0.05 ($-$)	0.60	0.09	0.15	0.26

Soil nutrient characteristics in the chronosequence fields

To determine potential changes in nutrient composition during old-field succession and its relationship with *J. vulgaris* cover, from each chronosequence field ten soil samples were collected. Three or four individual soil samples (3 cm diameter and 15 cm depth) were combined so that there were three homogenized soil mixtures per field. This resulted in 30 mixtures (10 fields $\times$ 3 combined soil samples), which were dried at 40°C for three days. Soil mineral N was extracted by shaking 10 g (dry weight) soil with 50 ml 1 M KCl for 2 h. NH_4^+-N and NO_3^--N were determined colorimetrically in the KCl extract. Their concentrations were summed to express total mineral N. C:N-ratio was measured on a NC soil analyzer. pH was measured in 2:5 dry soil:water suspensions. The percentage organic C was determined according to Nelson and Sommers (1982) and available P according to Olsen et al. (1954) and color intensity was measured at 720 nm.

Plant–soil feedback experiment

In a plant–soil feedback experiment we examine the effect of the soil community on *J. vulgaris* biomass production. We conducted this greenhouse experiment with soil that originated from the old-fields. The greenhouse experiment consisted of two growth phases. The growth effects in the first (conditioning) phase of the experiment reflect the current net activity of pathogens, symbionts and decomposer organisms in the field, whereas the effects in the second (feedback) phase of the experiment represent the net effects of these groups of soil biota that may develop after *J. vulgaris* has been grown in the soil. For the conditioning phase, seeds were collected from field 12 (Table A1), surface sterilized (1 min in 0.1% chloride solution and rinsed) and germinated on glass beads. *Jacobaea vulgaris* plants were grown in 0.9–l pots filled with 1.2 kg soil (based on dry weight). The soil was a mixture of 6:1 sterilized bulk soil and field soil. To obtain sterilized bulk soil, soil (approximately 750 kg) was collected from field 12 from 5–20 cm below the

soil surface, sieved using a 0.5 cm mesh, homogenized, and sterilized by gamma irradiation (>25 KGray gamma irradiation, Isotron, Ede, the Netherlands). Field soil was collected by taking approximately 150 soil cores (3 cm diameter and 15 cm depth) randomly from each of the ten fields in July 2007. Soil of each field was sieved (0.5 cm mesh size) and homogenized. Control plants were grown in sterilized bulk soil inoculated with an autoclaved (three consecutive days, 20 min at 120°C) mixture of the inoculum soil of all ten fields.

In each pot three one-week-old seedlings were planted. Seedlings that died during the first week of the experiment were replaced. All treatments were replicated five times, which resulted in 55 pots in total (10 fields $\times$ 5 replicate pots + 5 sterilized control pots). Pots were placed randomly in a greenhouse at 70% RH, at 16 h 21°C (day) and 8 h 16°C (night). Natural day light was supplemented by metal halide lamps (225 μmol s^{-1} m^{-2} photosynthetically active radiation, 1 lamp per 1.5 m^2). Plants were watered every other day and initial soil moisture level (17% at soil dry weight basis) was re-set twice a week by weighing. After 10 weeks all aboveground biomass was harvested, oven-dried (70°C for five days) and weighed. The soil, including roots, in each pot was divided into four equal parts. From two parts the roots were gently rinsed and nematodes were extracted from a homogenized sub-sample of these rinsed roots (approximately 1 g based on dry weight), using the same procedure as for the field plants.

The other two parts per pot were used as inoculum for the second growth phase and were mixed in a 1:1-ratio with the sterilized bulk soil (640 g dry weight sterilized soil) to balance for potential nutrient deficiencies after the first growth phase. Also control plants were included. This control treatment is comparable to the control in the conditioning phase and is irradiated bulk soil which is inoculated with an autoclaved (three consecutive days, 20 min at 120°C) mixture of the inoculum soil of all ten fields. This new control was mixed in a 1:1-ratio with irradiated bulk soil, as was done for all pots in the feedback phase. All treatments were replicated five times, which resulted in 55 pots (10 fields $\times$ 5 replicate pots + 5 new sterilized

control pots). Three *J. vulgaris* seedlings were planted and seedlings that died during the first week of the experiment were replaced. After one week the seedlings were randomly thinned to two seedlings per pot. Plants were grown under the same conditions as during the conditioning phase. Plants were only grown for six weeks, as this length of time already showed clear feedback effects and any prolongation of the experiment would enhance the risk finding effects that are due to differences in nutrient availability or pot size limitation. Six weeks after transplanting, aboveground biomass was harvested, oven-dried (70°C for five days) and weighed. For both phases, the reduction in aboveground biomass production was calculated relative to the sterile control of that phase.

Soil biotic community

To identify potential agents of the plant–soil feedback that affect abundance and performance of *J. vulgaris* plants in the old-fields, we determined the nematode and fungal community composition. Subsamples of the roots of the ten plants from each field were combined per field. Nematodes were extracted from the combined sample of approximately 2 g dry root mass using a mistifier and an extraction time of 50 h. Nematodes were heat-killed and fixed (35% formaldehyde diluted to 4%). Plant-feeding nematodes were identified to genus or species level according to Bongers (1988) and the total number of plant-feeding nematodes per gram dry root material determined. Fungal community composition of a sub-sample from the pooled and homogenized rhizosphere soil of the ten *J. vulgaris* plants per field was determined using PCR-based denaturing gradient gel electrophoresis (DGGE), using fungal-specific primers (Appendix 1).

J. vulgaris seed weight, germination and seed bank

To determine if seed weight or germination rates are related to the age of the field or the abundance of *J. vulgaris*, we measured seed weight and germination of plants from each of the ten fields. In November 2007 *J. vulgaris* seeds were collected from approximately 100 plants in each of the fields. Seeds were air-dried and the pappus was removed, per field 100 randomly chosen seeds were weighed individually. To examine germination, seeds were surface sterilized (1 min in 0.1% chloride solution and rinsed) and 25 seeds were placed on filter paper (9 cm diameter) in a petri dish with 2 ml demineralised water. There were five replicate petri dishes for each field. After 16 days at 16 h 21°C (day) and 8 h 16°C (night), the number of germinated seeds was determined.

The number of *J. vulgaris* seeds that emerged from the soil seed bank was determined for seven fields (fields 2A, 5B, 12, 13, 17, 22, 25). Soil cores (approximately 50 cores from each field, 3 cm diameter and 7 cm depth) were collected in January 2008. Soil from each field was homogenized and sieved (0.5 cm mesh size). Plastic containers of 12.5 × 17 × 6 cm (l × w × h) were filled with 750 g sterilized (gamma irradiation) bulk soil, which was collected from field 12. This layer of bulk soil was topped-up with 250 g (both based on dry weight) field soil. There were five replicate containers for each field, resulting in 35 containers in

total (7 fields × 5 replicates). The soil used in each container corresponded with approximately 35 cm² of field surface (one core equals 7.1 cm² and 51 g of dry weight soil). The containers were placed in a greenhouse at 70% RH and kept at 17% moisture content. Emerged seedlings were removed every four weeks for a total period of six months and the number of *J. vulgaris* seedlings was recorded. After the first three months, the containers were placed at 4°C for a period of three weeks to break dormancy of the remaining seeds, and then returned to the greenhouse. For each container, the cumulative number of *J. vulgaris* seedlings was calculated and converted to number of individuals per m².

Seedling establishment in turfs transplanted to a common garden

To determine the effect of the plant and soil community in the old-fields on seedling establishment, we conducted a common garden experiment. We determined seedling establishment during an entire growth season in intact turfs that were collected in the old-fields. In December 2008, 30 × 30 × 30 cm turfs were collected from fields 2A, 5B, 12 and 25. Six turfs were collected from each field, resulting in 24 turfs in total (4 fields × 6 replicates). The turfs were placed in plastic rings of 25 cm diameter, which were placed on root cloth in a common garden so that the soil surface of the turf leveled with the surrounding soil. The garden was situated at the Netherlands Inst. of Ecology, Heteren, the Netherlands. In each turf nine rows of nine seeds were placed at 0.5 cm below the surface and 2.5 cm apart. All seeds originated from field 12. For each turf, at the start of the experiment the percentage bare ground was recorded. In September 2009, after one growing season, the percentage established seedlings was determined.

Data analyses

All data were analysed using univariate (GenStat 12; Payne et al. 2008) or multivariate statistics (Canoco 4.55; Ter Braak and Šmilauer 2002).

Univariate analyses
Field data, plant life history traits and results from the feedback experiment were analysed using analysis of variance (ANOVA), with field identity as a fixed factor. Individual comparisons were based on a Tukey HSD post hoc test. Before conducting ANOVA, data were checked for homogeneity of variances using Cochran's, Hartley's and Bartlett tests ($p > 0.01$) and for normality by inspection of the normal-probability plot. To fulfill requirements of normality, soil nutrients (P and mineral-N) and biomass of individual *J. vulgaris* plants in the field were log-transformed, count data were square root-transformed and percentage data were arcsin-transformed prior to statistical analyses. Percentage germination, *J. vulgaris* cover, and seed weight were analysed using a non-parametric Kruskal–Wallis test.

Linear relationships between measurements in each field and field age were analysed using linear mixed models (REML). Regressions were based on all measurements per field, but with field identity as random factor and field age as a continuous factor. Non-linear relationships, i.e. the relationship

between time since abandonment and *J. vulgaris* cover in chronosequence fields and in the experimental fields were described by fitting a log-normal curve.

To compare how well the measured variables (abiotic and biotic soil and *J. vulgaris* characteristics) relate to declined *J. vulgaris* cover, effect sizes were calculated as R^2 values of the relationship between cover or density of *J. vulgaris* and the explaining variable. This was done with average values per field and for all fields excluding field 2A and 2B (where *J. vulgaris* cover was still in the build-up phase).

Multivariate analyses

Plant and fungal community compositions in the ten fields were analysed using multivariate statistics using CANOCO (Lepš and Šmilauer 2003). Significance in direct analyses were inferred by Monte Carlo permutation tests (999 permutations) using permutation tests with multiple samples from each field (e.g. quadrats, plants, soil samples) as split-plots and the field as a whole plot. Whole plots were permuted freely and split-plots were not permuted. The presence/absence data of the soil fungal banding profiles was analysed using unimodal multivariate analyses (CA, CCA). Detrended correspondence analysis was used to analyse plant community cover data (log (n + 1) transformed) of the fields, rare species that were present in less than three quadrats were excluded from these analyses.

Results

Comparison of the long-term field site and the chronosequence

A comparison of the *J. vulgaris* cover at the long-term experimental field and the chronosequence showed that the chronosequence was a good substitute of time. In both cases there was a hump-shaped temporal pattern of cover that peaked at about five years after initial colonization or cessation of agricultural practices (Fig. 1A–B). The temporal cover of *J. vulgaris* in the experimental field site and *J. vulgaris* cover in the chronosequence fields followed a log-normal pattern ($p < 0.001$, $R^2 = 0.48$, and $p < 0.001$, $R^2 = 0.88$, respectively).

Field soil feedback response

In the conditioning phase of the feedback experiment aboveground biomass was on average 20 per cent lower in soils with live field inoculum than in the sterilized control (Fig. 2). The magnitude of growth reduction differed significantly between soils collected from different fields ($F_{9,49} = 7.42$, $p < 0.001$). Biomass reduction in the conditioning phase of the experiment did neither correlate with the age of the field from which the soil originated ($F_{1,8} = 0.02$, $p = 0.88$) nor with *J. vulgaris* cover in that field ($F_{1,8} = 0.27$, $p = 0.61$). However, there was a positive relationship between the number of *J. vulgaris* plants in the field (Fig. 1c) and the biomass reduction observed in the soil collected from that field. This relationship was significant only when we excluded field 13 ($F_{1,7} = 5.45$, $p = 0.05$, $R^2 = 0.44$; Fig. 3A). Opposite to all other soils, field 13 had a different history of less intensive use. This field had been used for grass seed production during the last decennia before cessation of agricultural practices, whereas the other soils had been used for intensively growing arable crops.

In the feedback phase, where plants were grown in soil in which *J. vulgaris* had been grown before, biomass reduction did not differ among fields ($F_{9,49} = 1.94$, $p = 0.08$; Fig. 2). However, aboveground biomass production was on average 70% lower in the conditioned soils than in the sterilized control (Fig. 2). There was no correlation between the strength of the feedback effect and field age or *J. vulgaris* cover in the field from which the soil originated ($F_{1,8} = 1.26$, $p = 0.29$; $F_{1,8} = 2.71$, $p = 0.14$, respectively). However, there was a negative relationship between growth reduction in the feedback phase and rosette size of the vegetative plants in the field the soil was collected from ($F_{1,8} = 5.41$, $p = 0.04$, $R^2 = 0.40$; Fig. 3B). This relationship was stronger when we excluded field 13 ($F_{1,7} = 8.60$, $p = 0.02$, $R^2 = 0.55$).

Common garden and seed bank experiment

In the common garden experiment, after one growth season the establishment rate of *J. vulgaris* varied significantly between fields ($F_{3,19} = 18.2$, $p < 0.001$) and establishment was highest in the two youngest fields (Fig. 4A). There was a
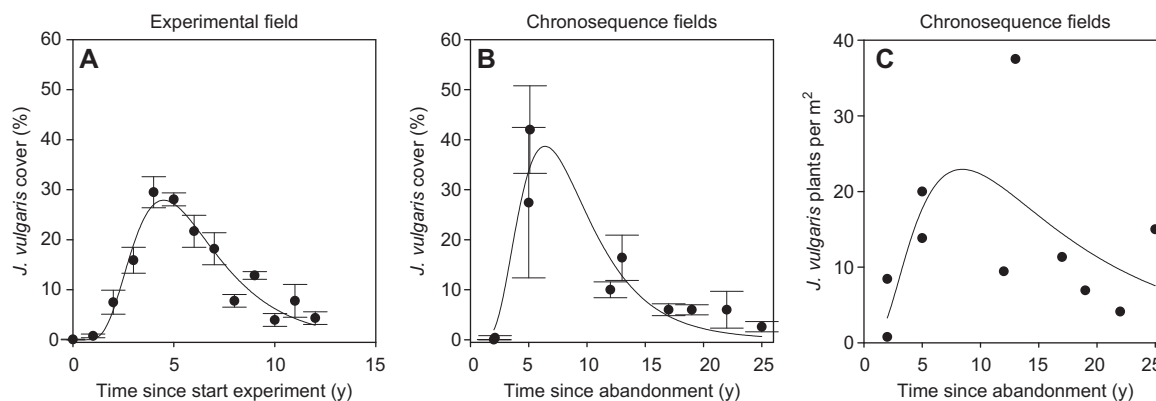

Figure 1. *Jacobaea vulgaris* population characteristics in a long-term experimental field site and in old-fields taken out of production between two and 25 years ago. Mean ($\pm$SE) (A) *Jacobaea vulgaris* cover in the experimental field site; and (B) in fields of the chronosequence, and (C) number of *J. vulgaris* plants per m² in the chronosequence. The line represents the estimated relationship based on a log-normal fit.
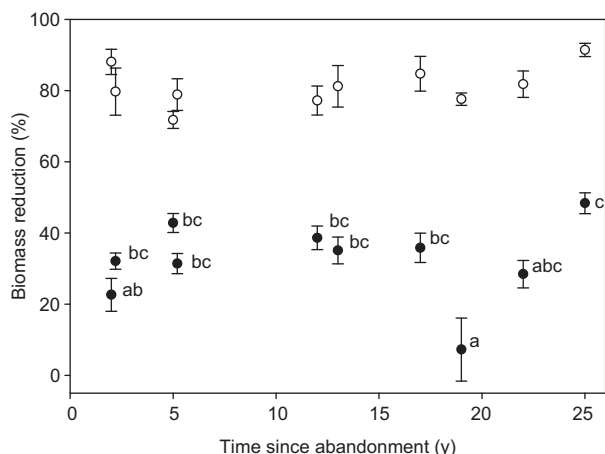
Figure 2. Percentage reduction of aboveground *Jacobaea vulgaris* biomass production in a greenhouse plant–soil feedback experiment, relative to biomass produced in sterile control soil. Means (±SE) are shown for biomass production in phase 1 (closed circles) and phase 2 (open circles). Different letters indicate significant differences (p < 0.05) within phase 1, based on a Tukey's HSD post hoc test. There were no significant differences in phase 2. To avoid overlapping data points, fields 2B and 5A are moved forward by 0.2 year.

positive relationship between the percentage of bare ground at the start of the experiment and *J. vulgaris* establishment in the turfs ($F_{1,3}$ = 10.1, p = 0.05, R^2 = 0.46).

In the seed bank experiment the average number of emerged *J. vulgaris* seedlings varied between 0.8 to 7.2 individuals per container, corresponding to 230 to 2070 seedlings per m^2. Seedling density followed a log-normal pattern (Fig. 4B; p < 0.001, R^2 = 0.56). There was a significant relationship between the number of emerged *J. vulgaris* seedlings and *J. vulgaris* field cover ($F_{1,5}$ = 9.02, p = 0.03, R^2 = 0.64). However, we did not find a relationship between the number of emerged seedlings and number of *J. vulgaris* individuals per m^2 in the field ($F_{1,5}$ = 0.89, p = 0.39). This indicates that the number of propagules in the fields may not be limiting, but that other processes reduce the number of successful individuals.
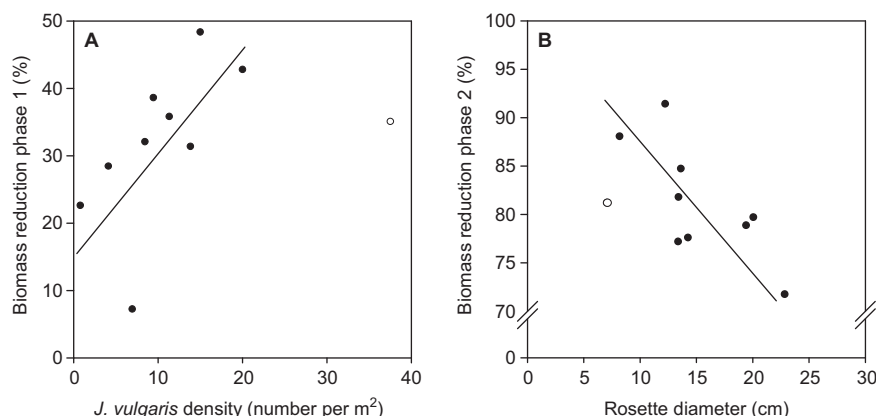
Identification of soil biota

The fungal community composition in the rhizosphere (according to DGGE; Fig. A1) could be significantly explained by field age (CCA: p = 0.04; 14.4%), but not by *J. vulgaris* cover (CCA: p = 0.55). The number of DGGE fungal bands in the rhizosphere soil of the field plants ranged from four (field 12 and 25) to 12 (field 2A and 5A).

In the roots of the field plants, more than 85% of all plant-feeding nematodes were identified as *Pratylenchus crenatus*. Nematode density decreased significantly with field age ($F_{1,8}$ = 14.2, p = 0.005, R^2 = 0.64; Fig. 5A). Other plant-feeding nematode species belonged to the genus *Filenchus*. *Pratylenchus crenatus* was also the most abundant plant-feeding nematode in the roots of the plants of the feedback experiment and represented more than 75 per cent of all plant-feeding nematodes. In the feedback experiment nematode densities differed significantly between fields (F = 10.2, p < 0.001, Fig. 5B), but they were not related to field age (p = 0.94, R^2 = 0.12).

Plant community composition and nutrients in the fields

A total of 71 plant species were recorded in the 50 quadrats in the ten fields. Plant community composition differed between fields and could be significantly explained by field age (CCA: p = 0.03, 8.5%; Fig. A2). Soil nutrients differed between fields (Table 1), but available phosphorous was the only element to decrease significantly with field age (Table 1).

J. vulgaris life history traits in relation to field age

The average number of *J. vulgaris* individuals per m^2 ranged from 0.8 to 37.5 (Fig. 1C). However, these numbers did not correlate with *J. vulgaris* cover in the field ($F_{1,8}$ = 1.73, p = 0.22, R^2 = 0.18). Individual plant height varied substantially between fields, and plants were tallest in the youngest fields (Table 2). The average shoot weight of the ten selected plants was significantly higher in field 5B than in the other fields and significantly lower in field 13 ($F_{9,89}$ = 2.31,
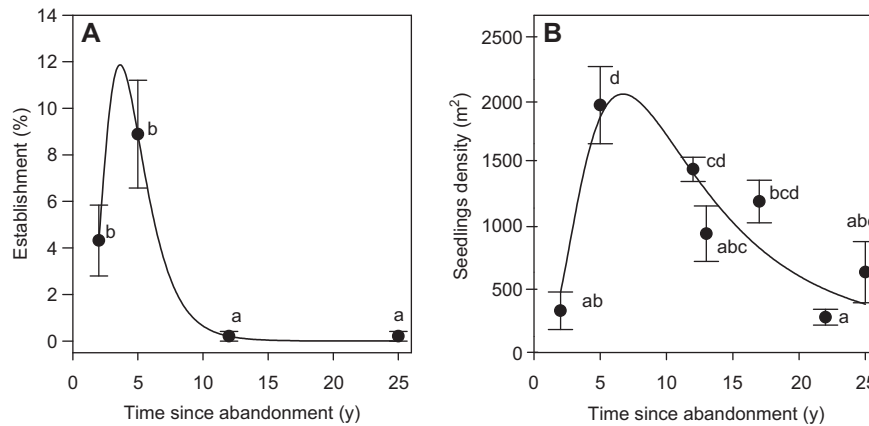

Figure 3. Relationship between (A) the number of *Jacobaea vulgaris* plants per m^2 in each field and aboveground biomass reduction in phase 1 of the feedback experiment ($F_{1,7}$ = 5.45, p = 0.05, R^2 = 0.44), and (B) relationship between aboveground biomass production in phase 2 of the feedback experiment and rosette size of vegetative field plants ($F_{1,7}$ = 8.60, p = 0.02, R^2 = 0.55). The line is the estimated relationship based on linear regression analysis excluding Field 13 (Results). Values for Field 13 are presented by open circles.

Figure 4. Mean (±SE) (A) *Jacobaea vulgaris* seedling establishment in turfs with intact vegetation in a common garden experiment, and (B) number of *J. vulgaris* seedlings in seed bank in soil from a chronosequence of old-fields taken out of production between two and 25 years ago. Within each panel, different letters indicate significant differences (p < 0.05) based on a Tukey's HSD post hoc test.

p = 0.02, Table 2). There was no correlation between shoot weight and field age ($F_{1,8}$ = 0.01, p = 0.91) or *J. vulgaris* cover ($F_{1,8}$ = 0.01, p = 0.92). Individual seed weight differed significantly between fields (p < 0.001; Table 2) and tended to decline with time since abandonment, but this effect was not significant due to the low seed weights in the two five-year-old fields. Percent germination also differed between fields (p = 0.002; Table 2), but did not correlate with field age ($F_{1,8}$ = 1.15, p = 0.31).

Effect sizes

Cover of *J. vulgaris* in the eight oldest fields was most strongly related to the number of *J. vulgaris* seedlings that emerged from the seed bank, the soil P content, and the C:N ratio of the soil. Plant density of *J. vulgaris* in these fields was most strongly related to the germination rates from seeds that were collected in these fields, and the percentage organic C and C:N ratio of the soil. The effect sizes of the other relationships between abiotic and biotic soil characteristics, and *J. vulgaris* life history traits and *J. vulgaris* cover and density in the 8 oldest chronosequence fields is given in Table 3.

Discussion

Jacobaea vulgaris cover showed a hump-shaped temporal pattern during old-field succession and cover peaked about five years after cessation of agriculture. This pattern occurred both in the experimental field and in the chronosequence, which validates the use of samples collected from the chronosequence fields as a space for time replacement (Johnson and Miyanishi 2008). In addition, the observed similarity in temporal cover of *J. vulgaris* in the experimental field site and in the chronosequence fields shows that this pattern is caused by robust mechanisms occurring in both systems. In a series of experiments we examined to what extent the hump-shaped pattern of *J. vulgaris* cover during old-field succession was related to plant–soil interactions, soil nutrients, seed availability and performance, and seedling establishment. We show that it is not one factor, but that a combination of processes is important. Below, we discuss the measured factors and their importance for the *J. vulgaris* cover in the chronosequence fields.

The conditioning phase of the plant–soil feedback experiment revealed that living soil from all fields caused a
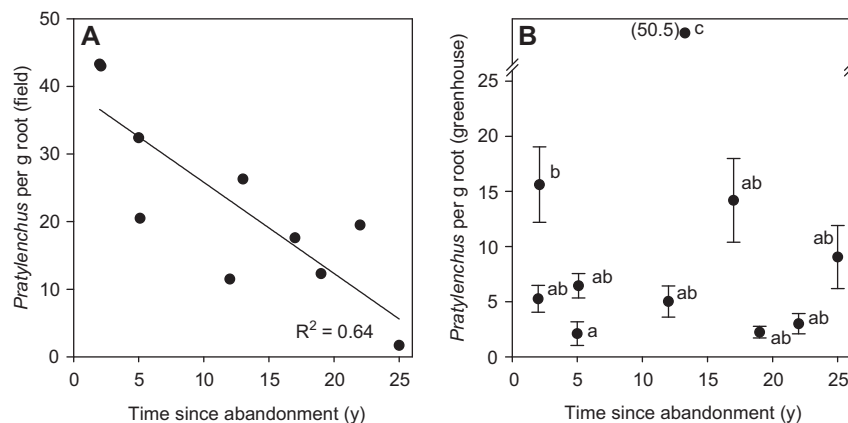

Figure 5. Relationship between time since abandonment and density per gram root of the plant feeding nematode species *Pratylenchus crenatus* in roots of *J. vulgaris* plants from (A) the field; and (B) the greenhouse plant–soil feedback experiment. Nematode numbers were determined in plants originated from or that were grown in soil from a chronosequence of old-fields. Means (±SE) are shown for greenhouse plants, different letters indicate significant differences (p < 0.05) based on a Tukey's HSD post hoc test. In (B) plants growing in soil from field 13 had a much higher nematode density than the other fields. The actual density for this field is presented between brackets.

Table 2. Life history traits of *Jacobaea vulgaris* in the chronosequence fields. Means (±SE) and p-values of ANOVA testing differences between fields are presented. Within columns, means followed by the same letter are not significantly different (p < 0.05) based on a Tukey HSD test. The outcome and direction of a linear mixed model (REML) with time since abandonment (age) as a continuous factor and field identity as a random factor (DF = 8) are also presented.

Field code	Aboveground biomass (g)	Seed weight (mg)	Germination (%)	Reproductive plant height (cm)
2A	8.8 ± 2.3 ab	0.246 ± 0.01 bc	61.2 ± 5.2 ab	86.6 ± 7.6 cde
2B	6.1 ± 2.2 ab	0.283 ± 0.01 c	70.4 ± 2.7 ab	100.8 ± 3.2 e
5A	3.4 ± 0.7 ab	0.226 ± 0.01 b	54.0 ± 8.9 a	43.9 ± 1.9 a
5B	19.5 ± 6.1 b	0.196 ± 0.01 ab	68.0 ± 2.5 ab	83.7 ± 6.3 de
12	7.0 ± 3.1 ab	0.249 ± 0.01 bc	60.0 ± 3.1 ab	62.8 ± 2.6 b
13	1.7 ± 0.6 a	0.239 ± 0.01 bc	86.4 ± 1.0 b	45.1 ± 2.2 a
17	9.0 ± 3.5 ab	0.242 ± 0.01 bc	62.8 ± 2.6 ab	67.8 ± 4.2 bcd
19	12.3 ± 4.8 ab	0.210 ± 0.01 ab	51.2 ± 4.8 a	65.6 ± 2.6 bc
22	7.6 ± 3.2 ab	0.178 ± 0.01 a	47.3 ± 6.4 a	64.5 ± 3.2 bc
25	7.9 ± 3.0 ab	0.216 ± 0.01 ab	56.9 ± 2.2 ab	64.5 ± 2.8 bc
p (fields)	0.02	<0.001	0.002	<0.001
p (REML ~ age)	0.91	0.12	0.28	0.35

reduction in biomass production (relative to sterilized soil) of *J. vulgaris* in the greenhouse. This effect became substantially more negative in the feedback phase, when the soil first had been conditioned by *J. vulgaris*. This shows there was a rapid development of a negative soil feedback in all fields independent of the age of the field, or the cover of *J. vulgaris* in that field. As plant–pathogen relationships may follow a density-dependent response (Augspurger 1983), we expected growth reduction by the soil community in the conditioning phase to be strongest in the fields with the highest *J. vulgaris* cover. However, in the feedback experiment, growth reduction in the conditioning phase was not related to *J. vulgaris* cover or density in the field the soil was collected from, except when

Table 3. Relationship between abiotic and biotic soil characteristics, and *Jacobaea vulgaris* life history traits and *J. vulgaris* cover and density in the 8 oldest chronosequence fields in which *J. vulgaris* cover declines. For each relationship the direction, positive (+) or negative (−), and R²-values are given. Significant relationships are indicated with *p < 0.05; ***p < 0.001.

		J. vulgaris field characteristics	
		Cover	Plants per m²
Abiotic soil characteristics	P	0.52* (+)	0.05 (−)
	% org. C	0.00	0.43 (−)
	C:N ratio	0.49* (+)	0.35 (+)
	min-N	0.31 (−)	0.29 (−)
	pH H₂O	0.06 (−)	0.00
Biotic soil characteristics	soil feedback phase 1	0.01	0.12 (+)
	soil feedback phase 2	0.28 (+)	0.00
	fungal composition CA 1	0.31 (−)	0.03
	fungal composition CA 2	0.01	0.15 (−)
	Pratylenchus (field)	0.35 (+)	0.22 (+)
J. vulgaris life history traits	seed weight	0.00	0.11 (+)
	germination	0.02 (+)	0.89*** (+)
	seedling density seedbank	0.56* (+)	0.05 (+)

we excluded field 13. This field responded in an idiosyncratic way, as it did in a previous study (van der Wal et al. 2009). The abiotic characteristics of this field are similar to the other fields, but this field has a history that differs from all other fields, because it has not been used as intensively for agriculture as most other fields. This is also the only field where the target plant species *Calluna vulgaris* established spontaneously almost immediately after agricultural practices were ceased (van der Wal et al. 2009). Typically, it takes many decades before *C. vulgaris* plants can be detected on former arable fields (Kardol et al. 2005). The idiosyncratic reaction of field 13 suggests that spatial variation in plant–soil interactions outdoors could be important as well.

The growth effects that we observed in the feedback phase of the greenhouse experiment are the result of the net effects of pathogens, symbionts and decomposer organisms in the soil that may develop following plant presence. Thus, our results show that in all fields, even in those that are at the youngest stages of succession, some latent net soil pathogen activity is present and that a more severe effect can develop in a period shorter than a growth season. The results from the feedback phase suggest that soon after soil has been colonized by *J. vulgaris* it will become less suitable for subsequent plant growth of this species. Once the soil of an entire field has become conditioned, newly establishing plants will become exposed everywhere to the negative soil feedback, provided that the agents causing the negative feedback persist for at least some years in the absence of *J. vulgaris*. When new individuals have to establish in conditioned soil this will reduce their performance and the possibilities for successful establishment. An alternative explanation for the negative soil effect could be the release of phytochemicals by *J. vulgaris*. However, in a previous study we found that soil conditioning by *J. vulgaris* has a positive effect on the performance of plant species that co-occur with *J. vulgaris* in ex-arable fields, which suggests that the observed effect is not due to allelochemicals (van de Voorde et al. 2011).

The composition of the fungal community in the rhizosphere soil from fields 5B, 12 and 25, as determined by DGGE, differed from the communities in the other fields. *Jacobaea vulgaris* biomass production in the conditioning

phase was also poorest in these soils, suggesting that soil fungi may be responsible for plant growth reduction (Bezemer et al. 2006). However, the DGGE patterns of fields 5B, 12 and 25 also differed from each other, indicating that soil fungi may not necessarily be the only agents reducing *J. vulgaris* biomass production, or that different fungal taxa may do the same job. Also plant-feeding nematodes can reduce plant performance. In the field the number of plant-feeding nematodes decreased over time, which is probably a direct result of the cessation of crop growing (Korthals et al. 2001). In the feedback experiment plant-feeding nematodes were almost absent in *J. vulgaris* roots, although diverse nematode communities were found in the soil of these old-fields (Kardol et al. 2005). The low nematode abundance in *J. vulgaris* roots could be due to the negative effects of pyrrolizidine alkaloids on plant parasitic nematodes (Thoden et al. 2009). A strong negative effect of plant-feeding nematodes on *J. vulgaris* performance and establishment is therefore not to be expected, although this needs to be confirmed in inoculation experiments. Unlike in the field, in the greenhouse experiment there was no relationship between time since abandonment of the field the soil originated from, and the number of nematodes that were found in the roots of the greenhouse plants. This could be because we used inoculated soil, which reduces the differences in soil nutrients between fields, which subsequently could reduce fluctuations in number of plant-feeding nematodes. Nutrient availability affects the PA concentrations in the roots of *J. vulgaris* (Hol et al. 2003), and this may also have influenced the number of plant-feeding nematodes. However, further studies are needed in order to reveal the exact mechanisms causing this pattern.

The negative plant–soil feedback effects may be amplified when later succession plant species colonize, as they will be disproportionally more competitive when their predecessor is reduced selectively by negative soil feedback (Kardol et al. 2007). Indeed, the common garden experiment with intact vegetation turfs showed that in older fields soil and vegetation conditions reduced seedling establishment. After one growth season, seeds of *J. vulgaris* had established better in turfs from young than from older fields, and there was a positive relationship between percentage bare ground in the beginning of the experiment and *J. vulgaris* establishment. This shows that besides the effect of the soil, the structure and openness of the surrounding vegetation are important characteristics that determine germination and seedling success of *J. vulgaris*, for example by influencing light availability and micro-climate (van der Meijden and van der Waals-Kooi 1979, Olff et al. 1994). Therefore, the reduced availability of bare ground and the increased competition with other plants in later stages of old-field succession can change establishment success. Thus, changes in bare ground availability and competition all may contribute to the pattern of population development of *J. vulgaris* during old-field succession by changing establishment success.

The number of emerging *J. vulgaris* seedlings from the seed bank corresponded with *J. vulgaris* cover in the fields, but was substantially higher than the number of *J. vulgaris* plants recorded in the field. The higher numbers of seedlings in the seed bank experiment per surface area can be explained by the soil disturbance that was applied during the seed bank experiment and by the absence of aboveground herbivores. However, the relatively high number of seedlings that emerged from the seed bank also suggests that in the field the availability of viable propagules is not limiting (Tilman 1997, Ozinga et al. 2005), but that the absence of disturbances (van der Meijden and van der Waals-Kooi 1979) or the presence of aboveground herbivores (Wilby and Brown 2001) may be limiting *J. vulgaris* abundance in the field. Our results suggest that only in the youngest fields, the number of seedlings may be limited by seed availability. These results therefore suggest that the initial increase in *J. vulgaris* cover from two- to five-year-old fields is due to increased seed availability. Seed availability in the seed bank and germination rates were both high in all fields, however, there was a strong relationship between germination rate and *J. vulgaris* density in the corresponding field. This positive relationship could mean that a substantial part of the seeds do not survive in the field, for example due to seed herbivory, and that germination rates are important for the remaining seeds. While seed weight did not appear to strongly contribute to the general trend in *J. vulgaris* population development during old-field succession, seed weight in both fields where *J. vulgaris* cover peaked was reduced compared to other fields. Reduced seed weight may have a negative effect on plant fitness and the competitive ability of these seedlings (Crawley and Nachapong 1985), thereby negatively affecting fitness of the next generation.

Also, nutrient availability can influence plant performance and plant community composition during succession (Huston 1994). Although there was a strong relation between the phosphorous content in the soil and *J. vulgaris* cover in the fields, it is not possible to directly relate these to each other, as the decrease of P is also directly related to time since abandonment (van der Wal et al. 2007). In addition, in our study nutrient availability was relatively high in all fields. Therefore, it is unlikely that limited nutrient availability is a direct cause of decline of *J. vulgaris* populations. In conclusion, we show that the boom-and-burst pattern of *J. vulgaris* population development is initially limited by propagule availability. In all stages of the chronosequence negative plant–soil feedback effects can be induced and the growth reduction in soils directly collected from field sites tends to relate positively to the density of *J. vulgaris* in the fields. However, this feedback effect is not operating alone, and soil nutrient status and *J. vulgaris* seed characteristics are also important for *J. vulgaris* cover on restored grasslands. Thus, a combination of multiple processes plays a role in determining the dynamics of plant populations along successional gradients, and these processes need to be considered within a community framework.

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Appendix 1

Supplementary information about DGGE analysis

Fungal community composition in each field was determined using PCR-based denaturing gradient gel electrophoresis (DGGE; Muyzer et al. 1993) of a sub-sample from the pooled and homogenized rhizosphere soil of ten *J. vulgaris* plants. DNA was extracted using a DNA isolation kit. DNA quantity and quality were assessed using spectrophotometer. The extracted DNA was amplified using the fungal-specific primers ITS1f and ITS4 (White et al. 1990). All amplification reactions were performed a thermal cycler in a volume of 25 µl and consisted of 15 µmol of each primer, approximately 50 µg of environmental template DNA, 2 U DNA polymerase, and the manufacturer's recommended nucleotide concentrations and buffer conditions. PCR amplification conditions were 5 min at 95°C, followed by 32 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and an extension step of 72°C for 10 min, followed by 10°C until further use. PCR product size and quantity were verified on a 1.5% agarose gel. PCR product (20 µl) was used for DGGE analyses, which were performed in 6% acrylamide gels with a gradient of 20–60% denaturant (100% denaturant =

7 mol l^{-1} urea with 40% formamide) using the DGene system. Gradient gels were topped with 10 ml of acrylamide containing no denaturant. Electrophoresis was carried out at 60°C for 10 min at 200 V, followed by 17 h at 80 V. Gels were stained with ethidium bromide for 20 min followed by destaining them for 20 min, prior to UV transillumination and digital photography using the ImaGo system. DGGE banding patterns were analysed using Imagemaster elite ver. 4.20 with rolling ball background subtraction, normalization, and band detection.

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Table A1. Characteristics of chronosequence fields. Field properties (name, year of abandonment and location) and plant community characteristics (diversity and species richness) of the fields in a chronosequence of old fields that were abandoned two to 25 years ago. Means (±SE) are shown for diversity and species richness, different letters indicate significant differences (p < 0.05) based on a Tukey's HSD post hoc test.

| Field code (age)	Field name	Year of abandonment	Latitude (ᵒN)	Longitude (ᵒE)	Diversity (H′)	Species richness
2A	Oud Reemst	2005	52.02	5.48	0.64 ± 0.13 a	3.0 ± 0.0 a
2B	Reyerskamp	2005	52.01	5.47	0.93 ± 0.08 ab	3.6 ± 0.4 ab
5A	Telefoonweg	2002	52.00	5.45	1.00 ± 0.19 ab	4.4 ± 0.4 b
5B	Assel	2002	52.12	5.49	1.21 ± 0.13 ab	8.6 ± 0.9 cde
12	Mossel	1995	52.03	5.45	1.38 ± 0.27 b	9.8 ± 0.7 e
13	Plantage Willem III	1994	51.59	5.31	1.35 ± 0.11 b	7.6 ± 0.7 cde
17	Nieuw Reemst	1990	52.04	5.47	1.09 ± 0.08 ab	7.0 ± 0.9 cd
19	Wolfhezerveld	1988	51.6	5.47	1.19 ± 0.10 ab	6.6 ± 0.7 c
22	Mosselse veld	1985	52.04	5.44	1.45 ± 0.13 b	9.4 ± 1.2 de
25	Dennenkamp	1982	52.02	5.48	1.30 ± 0.09 b	7.4 ± 1.3 cd
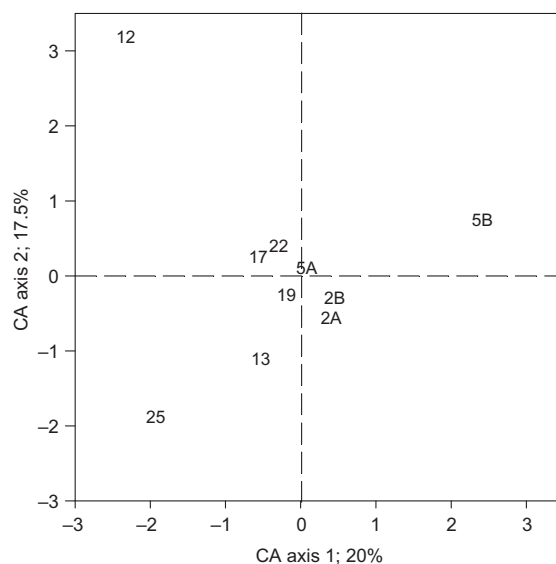
Figure A1. Soil fungal community composition in the chronosequence fields, based on DGGE analyses. Shown are the sample scores of an unconstrained unimodal canonical analysis (CA) of soil fungal community composition based on presence–absence data of DGGE bands.
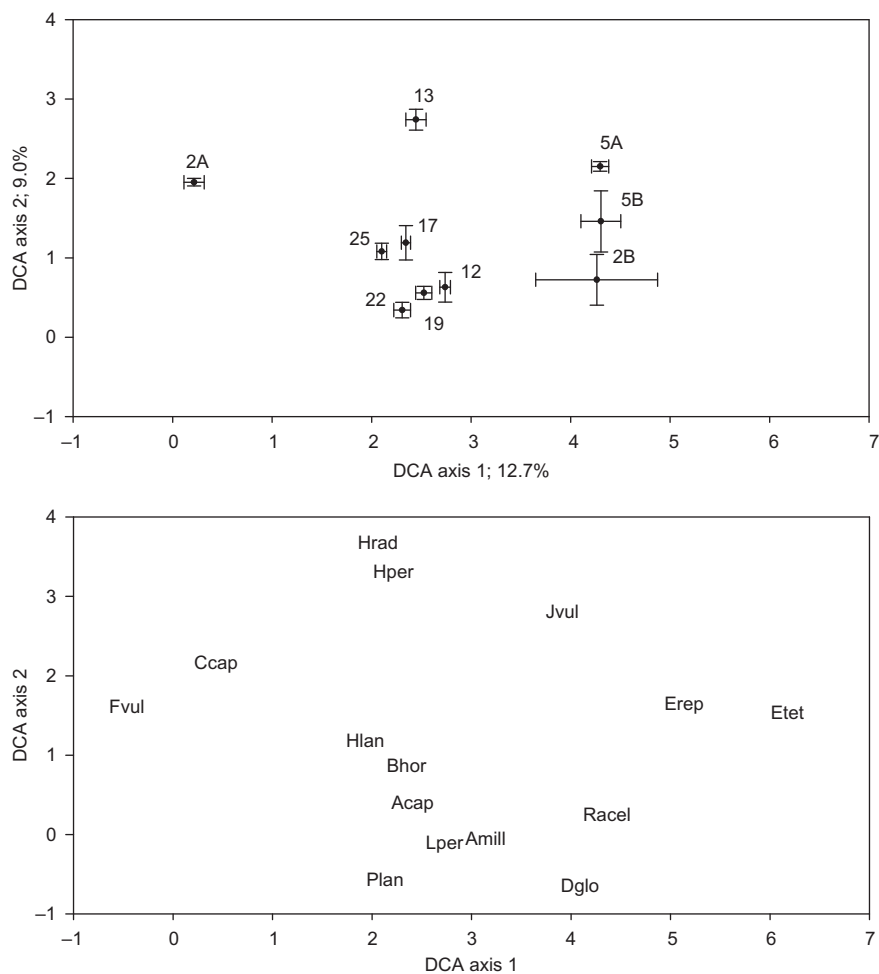

Figure A2. Plant community composition in the chronosequence fields. Shown are the results of a detrended correspondence analysis of mean sample scores (±SE) and species scores of the plant community composition in five quadrats in each field. Species cover data were log (n + 1) transformed and amount of explained variation by the first two DCA axes is given. Names of the 15 most contributing plant species are: Amill = *Achillea millefolium*, Acap = *Agrostis capillaris*, Bhor = *Bromus hordeaceus* subsp. *Hordeaceus*, Ccap = *Crepis capillaris*, Dglo = *Dactylis glomerata*, Erep = *Elytrigia repens*, Etet = *Epilobium tetragonum*, Fvul = *Filago vulgaris*, Hlan = *Holcus lanatus*, Hper = *Hypericum perforatum*, Hrad = *Hypochaeris radicata*, Jvul = *Jacobaea vulgaris*, Lper = *Lolium perenne*, Plan = *Plantago lanceolata* and Racel = *Rumex acetosella*.