Enhancement of Late Successional Plants on Ex-Arable Land by Soil Inoculations

Vanessa Carbajo1,2, Bowy den Braber1,3, Wim H. van der Putten1,4, Gerlinde B. De Deyn1*

1 Department of Terrestrial Ecology, Netherlands Institute of Ecology, Wageningen, The Netherlands, 2 Department of Ecology, Alcalá University, Madrid, Spain, 3 Nature Conservation and Plant Ecology Group, Wageningen University and Research Centre, Wageningen, The Netherlands, 4 Laboratory of Nematology, Wageningen University and Research Centre, Wageningen, The Netherlands

Abstract

Restoration of species-rich grasslands on ex-arable land can help the conservation of biodiversity but faces three big challenges: absence of target plant propagules, high residual soil fertility and restoration of soil communities. Seed additions and top soil removal can solve some of these constraints, but restoring beneficial biotic soil conditions remains a challenge. Here we test the hypotheses that inoculation of soil from late secondary succession grasslands in arable receptor soil enhances performance of late successional plants, especially after top soil removal but pending on the added dose. To test this we grew mixtures of late successional plants in arable top (organic) soil or in underlying mineral soil mixed with donor soil in small or large proportions. Donor soils were collected from different grasslands that had been under restoration for 5 to 41 years, or from semi-natural grassland that has not been used intensively. Donor soil addition, especially when collected from older restoration sites, increased plant community biomass without altering its evenness. In contrast, addition of soil from semi-natural grassland promoted plant community evenness, and hence its diversity, but reduced community biomass. Effects of donor soil additions were stronger in mineral than in organic soil and larger with bigger proportions added. The variation in plant community composition was explained best by the abundances of nematodes, ergosterol concentration and soil pH. We show that in controlled conditions inoculation of soil from secondary succession grassland into ex-arable land can strongly promote target plant species, and that the role of soil biota in promoting target plant species is greatest when added after top soil removal. Together our results point out that transplantation of later secondary succession soil can promote grassland restoration on ex-arable land.

Introduction

During the last century, in industrialized countries, species-rich grasslands have become rare due to land-use intensification and atmospheric deposition of nitrogen [1], [2]. These changes have promoted a select number of high productive plant species, causing the decline of many slow growing plant species that typify species rich grasslands [3], [4]. In order to counteract this decline a fraction of the arable land is being restored into semi-natural species-rich grasslands [5]. The (re)creation of these species-rich systems, however, requires the presence of specific abiotic and biotic conditions [5]–[7]. Even after re-establishment of meso- or eutrophic systems, conditions often remain favorable for early successional, fast growing species, whereas conditions are less conducive for late successional species because of high residual fertility and N deposition [1], [4], [8]. In order to overcome this constraint of excessive soil fertility, managers mow and remove hay [6], introduce herbivores that graze and concentrate nutrients [9], add carbon rich substrates which stimulates nutrient immobilisation by soil microbes [10], [11] or they remove the entire top soil [5], [7], [12].

The potential biotic constraints for biodiversity restoration are manifold, but to date the aspect of availability of species of target plant communities received most attention. The absence of late successional species from the seed bank and poor dispersal and colonization possibilities due to habitat fragmentation can clearly impede restoration of target plant communities [13], [14]. To overcome the limitation of absence of propagules of target plant species seed additions or spreading of hay containing seeds of desired plant species can be considered [15]–[17]. However, the availability of seeds of target plant species does not guarantee their establishment and there is growing awareness that also biotic soil properties may be of key importance for vegetation, and more generally, for biodiversity restoration [18], [19].

Theoretical and empirical studies show that soil biota can strongly affect the establishment, diversity and successional replacement of plant species in time series of land abandonment on grassland [20], [21] and arable land [20], [22], [23]. Soil communities consist of biota that can directly promote (e.g. mycorrhizal fungi) or suppress (e.g. root herbivores and pathogens) plant growth, and of biota that mediate these direct interactions by predation or influencing nutrient availability [24]. Compositions of soil communities are dynamic and change along secondary succession gradients. For example, bacterial biomass and abundances of plant-feeding nematodes tend to decrease and abundances of saprophytic and mycorrhizal fungi, as well as of...
omni- and carnivorous nematodes tend to increase after land abandonment [23], [25]–[27]. Given these transitions in soil communities it is crucial to determine whether and how the origin of soil biota, in relation to restoration history, matters for the promotion of late successional vegetation.

Impact of soil biota on plant communities is dependent on soil nutrient status [20], even to the extend that mutualism can turn into parasitism as frequently reported for mycorrhizal fungi at high soil P availability [29]–[31]. Plant growth promotion of late successional plants through soil inoculations is therefore more likely to occur in nutrient poor soil, such as soil after top soil removal, than in nutrient rich top soil. Moreover stimulation of plant growth by symbiotic soil biota is often larger when whole and diverse communities rather than when only specific taxa are used as inoculum [31]. In top soil the establishment of such introduced soil biota may be difficult given the high abundances of residing soil biota, so that new introductions might be more successful after top soil removal. Moreover the donor soil not only serves as inoculum source but it is also a good habitat for the desired soil biota so that the effect of soil inoculation is likely to increase with larger inoculum density.

Here we experimentally test whether soil inoculation could be a tool to improve restoration management strategies to restore species-rich grasslands. We tested three specific hypotheses: (1) the introduction of donor soil to promote target plant species is more successful after removal of the top layer of the arable receptor soil (2) donor soil from late successional or semi-natural grassland promotes late successional plants more than donor soil from early successional grassland (3) the impact of donor soil is dependent on dosage. We test these hypotheses under controlled conditions in a greenhouse in order to establish a proof of principle. In the case soil inoculation would work, those conditions may be studied in more detail under semi-natural and natural conditions in outdoor mesocosms and in the field.

**Results**

**Effects of donor and receptor soil on plant community biomass**

**Addition of donor soil in a 1:1 proportion.** Total plant community biomass (i.e. shoot plus root biomass) was significantly affected by addition of donor soil in a 1:1 proportion (F$_{6,77}$ = 93.71, P < 0.001), whereas the type of receptor soil had no main effect on plant community biomass (F$_{1,77}$ = 0.04, P = 0.85). However, the effect of 1:1 donor soil depended on whether organic or mineral arable soil was the receptor (donor x receptor soil interaction: F$_{6,77}$ = 6.06, P < 0.001) with generally a stronger response to donor soil additions in mineral than in organic receptor soil (Fig. 1a). Addition of donor soil resulted in an overall increased total plant community biomass, especially with donor soil from the later successional sites M2 and L1. However, inoculation of receptor soil with donor soil from the semi-natural field (L2) decreased total plant community biomass, especially in mineral receptor soil (Fig. 1a).

**Addition of donor soil in a 1:5 proportion.** Total plant community biomass was also affected by donor soil when it was added in smaller proportions (F$_{6,77}$ = 25.15, P < 0.001), and again an interaction with the type of receptor soil was found (donor x receptor: F$_{6,77}$ = 3.34, P < 0.01) while receptor soil had no main effect (F$_{1,77}$ = 0.56, P = 0.46) (Fig. 1b).

![Figure 1. Total plant community biomass in relation to soil treatments.](image)

Treatments are arable top soil (organic) or soil from the lower layer (mineral) mixed with a 1:1 (Fig. 1a) or 1:5 (Fig. 1b) proportion of donor soil from early (E1 and E2), mid (M1 and M2) or late (L1 and L2) successional restoration grasslands or without donor soil (None). Bars are means ± 1 SE, N = 6 for donor soils and N = 12 for ‘none’. Bars not sharing the same letter are significantly different at P < 0.05 with capital letters indicating main effect of donor soil, small case letters indicate effect of donor x receptor soil. doi:10.1371/journal.pone.0021943.g001
small proportions of donor soil stimulated total plant community biomass in a similar way as large proportions did: especially soil from later successional sites M2 and L1 enhanced plant community biomass while adding soil from the semi-natural field (L2) resulted in reduced plant community biomass, especially in mineral receptor soil (Fig. 1b).

Comparison between donor soil proportions

Across the treatments that received donor soil the proportion of donor soil addition significantly affected the response of the plant community biomass ($F_{1,115} = 50.13$, $P < 0.001$), but this effect also depended on the origin of the donor soil [proportion×donor soil interaction: $F_{3,115} = 9.69$, $P < 0.001$). Large additions of donor soil generally yielded more plant biomass than small additions, especially for additions with the later successional soils M1, M2 and L1 (Fig. 2).

Effects of donor and receptor soil on plant community diversity

Donor soil addition in a 1:1 proportion strongly affected the diversity of the plant communities when considering plant biomass distribution over the different species, illustrated by a significant effect on the Simpson’s evenness index (SIEI) ($F_{6,77} = 13.80$, $P < 0.0001$). This response to donor soil addition did not depend on the type of receptor soil ($F_{6,77} = 1.43$, $P = 0.21$) and receptor soil type did not affect the SIEI ($F_{1,77} = 2.04$, $P = 0.16$). Plant community evenness was promoted by donor soil from several origins (Fig. 3). Donor soil from the semi-natural grassland L2 strongly promoted the SIEI, and also soil from M1 improved SIEI, albeit to a lesser extend. When less donor soil had been added at a ratio of 1:5 plant community evenness was not affected by soil addition ($F_{6,77} = 1.59$, $P = 0.16$) and ranged from 0.213±0.006 (with E2) to 0.237±0.009 (with L2). In the treatments with the lower donor soil addition SIEI was significantly higher in mineral (0.234±0.005) than in organic (0.222±0.004) receptor soil ($F_{1,77} = 5.69$, $P = 0.019$).

Plant community relations with biotic and abiotic soil properties

The variation in the plant communities across all soil treatments could be explained for 56% by our measured set of abiotic and biotic variables, according to multivariate redundancy analysis (RDA) (Fig. 4). The first canonical axis explained as much as 51.6% and the second axis only 1.7% of the total variation. Tests of the significance of specific biotic and abiotic soil variables for plant community composition revealed that only four variables significantly contributed to the canonical axes (underlined variables in Fig. 4). These variables were, in order of diminishing importance: abundance of bacterivorous nematodes (24%, $F$-ratio = 52.55, $P = 0.002$), soil ergosterol concentration (23%, $F$-ratio = 73.63, $P = 0.002$), total nematode abundance (5%, $F$-ratio = 16.03, $P = 0.002$) and soil pH (1%, $F$-ratio = 3.83, $P = 0.036$). The RDA diagram also illustrates relations between individual plant species and abiotic and biotic soil properties, as well as relations between these soil properties. A positive relation with mineral nitrogen availability was apparent for *H. radica*, with soil P for *A. dioica*, with soil Mg, %OM and pH for *A. montana* and with nematodes for *C. rotundifolia*. The grass species *F. ovina* and *N. stricta* related negatively to soil P and K. Abundances of nematodes in most of the nematode feeding groups related positively to soil mineral nitrogen availability, while plant-feeding nematodes showed little relation to other factors, except to soil P levels.

Antennaria dioica response to soil inoculum in the main experiment

Total plant biomass production of *A. dioica* was significantly dependent on the composition of the field soil that was inoculated into the sterilised mineral donor soil. There was an effect of the type of donor soil ($F_{5,55} = 4.55$, $P < 0.01$) and receptor soil ($F_{1,55} = 12.19$, $P < 0.001$), and the effect of donor soil depended on the type of receptor soil ($F_{5,55} = 2.45$, $P < 0.05$). Generally *A. dioica* plants were larger when grown with soil inoculum composed of mineral receptor soil and later successional donor soil M2 or L1.
Antennaria dioica biomass was not related to mineral nitrogen availability at the start of the experiment ($r = 0.04$, $p = 0.7$).

Soil fungi in the main experiment

The ergosterol concentration, a measure of saprophytic soil fungal biomass, at the end of the main experiment was significantly affected by the receptor soil and the donor soil. When added in a large proportion donor soil had a stronger effect than receptor soil (donor $F_{6,77} = 174.1$, $P < 0.0001$, receptor $F_{1,77} = 70.7$, $P < 0.001$). However, when added in small proportion the impact of receptor soil was stronger than that of donor soil (donor $F_{6,77} = 50.9$, $P < 0.0001$, receptor $F_{1,77} = 102.8$, $P < 0.0001$). Overall soils with organic receptor soil had higher concentrations of ergosterol than soils with mineral receptor soil. Nevertheless the addition of later successional (M and L) donor soil increased ergosterol concentrations as compared to unmixed receptor soil, especially when the donor soil was added in large proportion to mineral receptor soil (Fig. 6).

Discussion

The restoration of degraded ecosystems may greatly benefit from using an integrated above-belowground approach because of the interdependency of both ecosystem components [18], [32], [33]. Recent studies demonstrate that early and late successional plant species are differentially impacted by feedbacks with soil biota; early successional plant species are reduced and later successional plant species promoted by soil biota [22], [23]. Therefore, in theory late successional plants could be promoted in recently abandoned arable land by combined introduction of target plants and field soil from late successional fields. Yet to date only few studies tried to bring this in practice, probably because of the many open questions that still need to be answered in order to make results of soil additions more predictable [18], [32], [33]. We examined three key questions with respect to the promotion of late

Figure 3. Simpson’s evenness index (SIEI) and plant species proportional shoot biomass in response to soil treatments. Numbers above the bars are SIEI values. Treatments as in Fig. 1a. Bars not sharing the same letter are significantly different for SIEI at $P < 0.05$ (donor soil main effect).
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Figure 4. RDA diagram of plant species shoot biomass in relation to initial soil characteristics of the 26 soil treatments (24 mixtures and unmixed mineral and organic arable soil). Fine dotted arrows are abiotic and coarse dotted arrows are biotic characteristics. $Hr =$ Hypochaeris radicata, $Fo =$ Festuca ovina, $Cr =$ Campanula rotundifolia, $Am =$ Arnica montana, $Ns =$ Nardus stricta, $Ad =$ Antennaria dioica. $bf =$ bacterial feeders, $pf =$ plant+root-hair feeders, $hf =$ fungal feeders, $ca/om =$ carnivores+omnivores, total nema = all nematodes.
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successional target plant species in abandoned arable soil by soil inoculation: 1) are donor soil additions more effective after top soil removal, 2) is donor soil origin (with respect to restoration history) of key importance and 3) are the responses to donor soil additions dose dependent?

In answer to our first question, we found that the impact of donor soil addition on plant growth was generally strongest when soil was added to the mineral soil, which becomes exposed following top soil removal. Donor soil addition to the organic top soil was effective as well, but less than in the case of mineral soil. We expected these results based on previous findings that impacts of soil biota are stronger and positive with reduced nutrient availability [28], [30], [31]. The stronger impact of donor soil addition to mineral soil could be due to the rudimentary soil food web in the mineral receptor soil, which may be less competitive towards newly introduced soil biota. In the additional experiment we found confirmation of this idea, because plant growth was stimulated more by adding donor soil from later successional sites to mineral receptor soil than to organic receptor soil. In the field, also some other limitations may need to be controlled in order to further enhance soil biota establishment, for example soil moisture level [34].

In our experiments competition with the seed bank was eliminated by removing spontaneously emerging seedlings, but there were notably fewer weeds in the treatments with mineral than with organic receptor soil. In the field reduced competition with weedy species after top soil removal can provide great benefits to target species [7], [17], yet also after top soil removal

Figure 5. *Antennaria dioica* total dry biomass (mg dw/pot) in relation to soil inoculum from the main experiment. doi:10.1371/journal.pone.0021943.g005

Figure 6. Soil ergosterol concentration (mg/kg soil dw) at the end of the main experiment in relation to the origin and proportion of donor and receptor soil. Significant differences of donor soil addition compared to no addition (none) within receptor soil and its proportion are indicated by * (organic 1:1), ◦ (mineral 1:1) + (organic 5:1), + (mineral 5:1) with significance $P<0.01$ for double symbols and $P<0.001$ for triple symbols and $P = 0.06$ for (*). Horizontal dotted lines indicate the ergosterol concentrations in the receptor soils without added donor soils. doi:10.1371/journal.pone.0021943.g006
restoration of appropriate abiotic conditions is essential in order to make the receptor soil conducive for introductions of desired plants and soil biota [18], [34].

The answer to our second question was positive: the origin of the donor soil was the key factor driving the plant community responses. The largest biomass was produced with soil from late successional grassland and the most even plant community composition with soil from semi-natural grassland. These differences in plant community responses can be explained by especially biotic characteristics of the donor soils, such as ergosterol concentration and abundances of bacterial feeding nematodes. Although we do not have a detailed overview of the soil pathogens and mutualists that can strongly contribute to the responses [22], [23], our results do indicate that differences in soil community composition were at play. Moreover, the ergosterol concentrations at the end of the experiment still depended strongly on the soil treatments suggest that the addition and origin of the donor soil resulted in different biotic communities throughout the experiment. It is noteworthy that depending on soil origin, plant community biomass or evenness was promoted. Our results illustrate the range of possible outcomes depending on interactions between soil biotic and abiotic properties [35]. Soil from semi-natural grassland was extremely poor in phosphorus, which likely incurred larger carbon allocation to mycorrhizal fungi [31]. Such carbon cost may have been disproportionately larger for the dominant species so that it promoted plant community evenness. On the other hand, higher sensitivity to soil pathogens at low phosphorus levels [36], and especially of the dominant species H. radicata, remains an alternative explanation. The high abundances of plant-feeding nematodes in the semi-natural field indicate that pathogen pressure may have been relative high in that soil, although we found mostly plant associated/root-hair feeding nematodes which are thought to cause considerable less plant damage than the real parasitic nematodes [44]. The RDA analysis indeed indicates that the abundances of plant-feeding nematodes in the main experiment did not contribute to explain the variability in the biomass of the plant species. Biomass enhancement in soils inoculated with soil from later successional grasslands could be attributed to a soil food web where plant growth promoting biota counterbalanced negative impacts of other biota in the soil inocula.

Finally, we found that the plant community responses depended on the amount of donor soil added. Addition of soil in a 1:1 proportion to arable receptor soil had a stronger impact on all response variables than adding soil in a proportion of 1:5. Compared to the control the increase in biomass with L1 donor soil was about 100% with 1:1 addition and 33% with 1:5 addition, suggesting that the decline of biomass due to diminishing soil inoculum is not linear with the proportion of soil added. In contrast, the decrease in plant community biomass with L2 was similar for both proportions, while plant community evenness was significantly altered only by large and not by small additions of donor soil. These differential impacts may be attributed to responses caused by different biota that are more or less density dependent in their effects. Responses where rare biota play an important role will then be stronger affected by dose than responses caused by naturally abundant and easy transferable biota [37].

Overall, our work shows that inoculations of later successional soil into ex-arable land can promote the establishment of target plant species, as well as plant community evenness, and this may be most effective after top soil removal. We do recognize that soil from later successional sites is precious and it is not our intention to advocate harming bio-diverse sites for the benefit of restoring degraded sites. Therefore it will be crucial to develop ways of applying the soil introductions such that they are as effective as possible (i.e. needing as little inoculum as possible for maximal success of establishment of target species). The creation of hot spots could be a good approach whereby the precious donor material is introduced locally together with target plants. This approach may require that the inoculum is as intact as possible with as little competition from the residing biota as possible (e.g. after top soil removal). An approach along these lines was recently applied, with success, by Middleton and Bever [23], although the biodiversity of the transplants may decline when the receptor fields are not suitable for taking up the soil biota from the transplants [34].

Conclusions

Our results contribute to a new perspective for ecosystem restoration management. Current restoration tools tend to be limited to manipulations of soil fertility by top soil removal, grazing and hay making [7], [10] and additions of seeds [15]–[17]. We show that biomass production of late successional plant species on ex-arable land can be promoted profoundly by inoculation with field soil from grasslands of older successional age. We demonstrate that under controlled conditions the origin of donor soil is of greater importance than top soil removal but top soil removal can provide additional benefits. Moreover, responses are dependent on the added dose of donor soil. Now, field tests are needed in order to establish the impact of soil inocula under outdoor conditions, which can be constrained by many more factors that have been controlled in the greenhouse [34].

Materials and Methods

Soil origins and properties

All fields that served as source for donor or receptor soil are located on sandy or sandy loam glacial deposits in the central part of the Netherlands (Table 1). All soils were collected end of November 2009. The six grasslands that served as donor soils, were selected from a grassland restoration chronosequence as used by Kardol et al. [22] such that they could be grouped into roughly three age categories: E1 and E2 were considered early successional and had been under restoration for 5 years., M1 and M2 were considered mid successional being under restoration between 25 and 30 years, whereas L1 and L2 were designated as late-successional fields with L1 being under restoration since 41 years, and L2 being a semi-natural grassland.

In the arable field top soil was collected from the upper 15 cm layer, and mineral soil from the 50–65 cm layer. This soil was collected from an area of 4×1 m, sieved and homogenized, and top soil and mineral soil were kept separately throughout the further processing. In each of the donor grasslands soil was collected as five randomly distributed turfs of 30×30 cm (lxw) and 15 cm deep. Five random samples were collected from an area of 50×50 m², minimally 20 m from the field edge. Per field site, soil turfs were bulked, sieved (mesh size 1 cm) to remove most of the roots, stones and soil macrofauna, and homogenized. Soil abiotic and biotic parameters were determined on soil subsamples at the start of the experiment (see below and Tables 2, 3, 4, 5).

Plants

We planted plant communities consisting of Antennaria dioica (L.) Gaertn., Aruncus montana (L.), Carex panicea, Retama rufina (L.), Festuca ovina* tenuifolia (Sibth.) and Nardus stricta (L.), all species that belong to the target plant community Gentio pneumonanthes-Nardetum [38] in the main experiment and only A.
**Table 1.** Codes of field sites, time since abandonment and plant association.

| Code | Site Description | Field age (years) | Lat. | Long. | Plant association\(^1\) |
|------|------------------|-------------------|------|-------|------------------------|
| ORG  | Arable field near Reijerskamp | organic layer | Arable site | 52.02 | 5.77 | Wheat |
| MIN  | Arable field near Reijerskamp | mineral layer | Arable site | 52.02 | 5.77 | Wheat |
| E 1  | Oud-Reemst | 5 | 52.04 | 5.80 | 16Bc1- Lolio-Cynosuretum |
| E 2  | Reijerskamp | 5 | 52.01 | 5.78 | 16Bc1- Lolio-Cynosuretum |
| M 1  | Dennenkamp | 28 | 52.03 | 5.80 | 14Bb - Plantagini-Festucion |
| M 2  | Mosselsche Veld | 25 | 52.07 | 5.74 | 31Ba1 - Echo-Verbascetum typicum |
| L 1  | Boersbos | 41 | 52.06 | 6.00 | 19Aa1 - Galio hercynici-Festucetum ovinae |
| L 2  | Leempetten | Semi-natural site | | 52.27 | 5.73 | 19Aa2 - Gentiano pneumonanthes-Nardetum |

Field age = years since abandonment. Lat. = Latitude (\(^\circ\)N), Long. = Longitude (\(^\circ\)E).

\(^1\)According to Schamineé et al. [38].

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**Table 2.** Abiotic characteristics of unmixed field soils at the start of the experiment.

| Soil | \(\text{NO}_3\) (mg.kg\(^{-1}\)) | \(\text{NH}_4\) (mg.kg\(^{-1}\)) | Olsen-P (mg.kg\(^{-1}\)) | Total P (mg.kg\(^{-1}\)) | K (mg.kg\(^{-1}\)) | Mg (mg.kg\(^{-1}\)) | Na (mg.kg\(^{-1}\)) | pH | % OM | Soil texture | % sand | % silt | % clay |
|------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----|----|----------------|--------|--------|--------|
| ORG  | 6.51            | 0.17            | 124             | 992             | 88.2            | 91.6            | 9.1             | 6.3 | 6.4 | 69.1           | 29.3   | 1.6    |
| MIN  | 5.65            | 0.00            | 85              | 575             | 78.5            | 71.9            | 6.4             | 6.4 | 5.5 | 69.9           | 28.7   | 1.4    |
| E 1  | 4.15            | 0.18            | 127             | 1004            | 87.1            | 82.3            | 7.6             | 6.1 | 6.0 | 74.8           | 23.6   | 1.6    |
| E 2  | 2.85            | 0.61            | 105             | 781             | 63.2            | 57.3            | 2.4             | 6.3 | 4.9 | 69.5           | 28.9   | 1.6    |
| M 1  | 5.33            | 0.42            | 35              | 237             | 27.7            | 41.8            | 9.5             | 5.5 | 4.5 | 84.8           | 15.1   | 0.1    |
| M 2  | 4.42            | 0.48            | 97              | 517             | 28.2            | 34.1            | 15.7            | 5.3 | 4.7 | 76.7           | 22.7   | 0.6    |
| L 1  | 7.58            | 0.69            | 55              | 207             | 30.2            | 19.6            | 11.8            | 4.0 | 5.0 | 80.6           | 19.4   | 0.0    |
| L 2  | 0.00            | 0.40            | 0.1             | 32              | 34.1            | 70.5            | 19.4            | 5.3 | 8.4 | 65.5           | 33.3   | 1.2    |

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or L1) and 1/12 receptor field soil (organic or mineral). The rest (5/6 parts) of the soil in each treatment was sterilised mineral receptor soil. To test plant growth response to the soil treatments single individuals of the same plant species were used, in order to avoid plant competition effects interfering with responses of the focal species to the soil biota. As focal plant species A. dioica was chosen because it is in decline (red list species) and because it was not the dominant species in the plant communities in the main experiment. A single seedling of the target plant species Antennaria dioica was planted in each of the 72 containers filled with 300 g of

| Soil | Ergosterol (mg·kg⁻¹) | Nematodes (per 100 g soil dw; n = 2) | Total | Bacterial | Plant | Fungal | Omni/Carnivores |
|------|----------------------|-------------------------------------|-------|-----------|-------|--------|-----------------|
| ORG  | 0.80±0.01            | 1396±181                           | 747±87| 496±74| 70±22| 83±2   |
| MIN  | 0.45±0.01            | 682±4                               | 259±27| 343±40| 48±14| 31±2   |
| E1   | 0.99±0.01            | 2471±47                             | 1109±26| 1017±89| 200±7| 145±9  |
| E2   | 0.72±0.01            | 2277±123                            | 971±84| 483±66| 184±36| 639±63 |
| M1   | 2.94±0.01            | 3724±170                            | 2559±163| 581±127| 218±98| 365±37 |
| M2   | 2.96±0.01            | 8107±460                            | 5998±95| 1673±189| 595±69| 365±16 |
| L1   | 1.13±0.01            | 5908±57                             | 3928±204| 1431±48| 291±69| 258±30 |
| L2   | 7.99±0.01            | 5189±58                             | 1897±79| 2612±1| 525±4| 156±18 |

For nematodes abundances are given for their total and per feeding group (bacterial, plant, fungal feeders, omni- and carnivores).

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| Soil | Proportion | NO₃ (mg·kg⁻¹) | NH₄ (mg·kg⁻¹) | Olsen-P (mg·kg⁻¹) | Total P (mg·kg⁻¹) | K (mg·kg⁻¹) | Mg (mg·kg⁻¹) | Na (mg·kg⁻¹) | pH | % OM |
|------|------------|---------------|---------------|-------------------|-------------------|-------------|-------------|-------------|-----|------|
| ORG  | None       | 6.51          | 0.17          | 124               | 992               | 88.2        | 91.6        | 9.1         | 6.3 | 6.4  |
| ORG  | E1 1:5     | 6.11          | 0.18          | 124               | 994               | 88.0        | 90.0        | 8.9         | 6.2 | 6.3  |
| ORG  | E1 1:1     | 5.33          | 0.18          | 125               | 998               | 87.7        | 86.9        | 8.4         | 6.2 | 6.2  |
| ORG  | E2 1:5     | 5.90          | 0.25          | 120               | 957               | 84.0        | 85.8        | 8.0         | 6.3 | 6.1  |
| ORG  | E2 1:1     | 4.68          | 0.39          | 114               | 887               | 75.7        | 74.4        | 5.8         | 6.3 | 5.6  |
| ORG  | M1 1:5     | 6.31          | 0.22          | 109               | 867               | 78.1        | 83.3        | 9.2         | 6.1 | 6.1  |
| ORG  | M1 1:1     | 5.92          | 0.30          | 79                | 615               | 57.9        | 66.7        | 9.3         | 5.9 | 5.4  |
| ORG  | M2 1:5     | 6.16          | 0.23          | 119               | 913               | 78.2        | 82.0        | 9.9         | 6.1 | 6.1  |
| ORG  | M2 1:1     | 5.46          | 0.33          | 110               | 755               | 58.2        | 62.8        | 11.4        | 5.8 | 5.5  |
| ORG  | L1 1:5     | 6.69          | 0.26          | 112               | 862               | 78.5        | 79.6        | 9.6         | 5.9 | 6.1  |
| ORG  | L1 1:1     | 7.04          | 0.43          | 89                | 601               | 59.2        | 55.6        | 10.5        | 5.1 | 5.7  |
| ORG  | L2 1:5     | 5.42          | 0.21          | 103               | 832               | 79.2        | 88.0        | 10.9        | 6.1 | 6.7  |
| ORG  | L2 1:1     | 3.25          | 0.28          | 62                | 512               | 61.1        | 81.0        | 14.3        | 5.8 | 7.4  |
| MIN  | None       | 5.65          | 0.00          | 85                | 575               | 78.5        | 71.9        | 6.4         | 6.4 | 5.5  |
| MIN  | E1 1:5     | 5.40          | 0.03          | 92                | 646               | 80.0        | 73.6        | 6.6         | 6.3 | 5.6  |
| MIN  | E1 1:1     | 4.90          | 0.09          | 106               | 789               | 82.8        | 77.1        | 7.0         | 6.2 | 5.8  |
| MIN  | E2 1:5     | 5.19          | 0.10          | 88                | 609               | 76.0        | 69.5        | 5.8         | 6.4 | 5.4  |
| MIN  | E2 1:1     | 4.25          | 0.30          | 95                | 678               | 70.8        | 64.6        | 4.4         | 6.3 | 5.2  |
| MIN  | M1 1:5     | 5.60          | 0.07          | 76                | 519               | 70.0        | 66.9        | 6.9         | 6.2 | 5.3  |
| MIN  | M1 1:1     | 5.49          | 0.21          | 60                | 406               | 53.1        | 56.8        | 8.0         | 5.9 | 5.0  |
| MIN  | M2 1:5     | 5.45          | 0.08          | 87                | 565               | 70.1        | 65.6        | 7.6         | 6.2 | 5.4  |
| MIN  | M2 1:1     | 5.04          | 0.24          | 91                | 546               | 53.4        | 53.0        | 10.1        | 5.8 | 5.1  |
| MIN  | L1 1:5     | 5.97          | 0.12          | 80                | 514               | 70.5        | 63.2        | 7.3         | 6.0 | 5.4  |
| MIN  | L1 1:1     | 6.62          | 0.35          | 70                | 392               | 54.4        | 45.7        | 9.1         | 5.2 | 5.2  |
| MIN  | L2 1:5     | 4.71          | 0.07          | 70                | 484               | 71.1        | 71.7        | 8.6         | 6.2 | 6.0  |
| MIN  | L2 1:1     | 2.83          | 0.20          | 42                | 303               | 56.3        | 71.2        | 12.9        | 5.8 | 6.9  |
the soil mixtures with a moisture content of 20\%(w:w). Seedlings were planted three weeks after their germination and harvested after ten weeks of growth. The plants were grown in a greenhouse under the same controlled conditions as the plant communities of the main experiment.

**Measurements**

**Plants.** After 4 months of growth in the main experiment and 2.5 months of growth in the additional experiment, shoots and roots of each plant species were collected, dried to constant weight at 70°C and weighed. The reported total biomass comprises shoot plus root biomass. From each pot of the main experiment, a 300 g soil subsample was collected for the analysis of soil abiotic and biotic characteristics.

**Abiotic soil characteristics.** Soil mineral content was determined using sieved (4 mm mesh) fresh soil of all 8 unmixed field soils before the experiment and of all 168 pots at the end of the main experiment. Mineral N was extracted from soil subsamples (10 g dry weight eq.) by shaking in 50 ml 1 M KCl for 2 h, and filtering through a Whatman filter. The concentrations of NH$_4$ and NO$_3$ in the filtrate were determined colorimetrically using Traacs 800 auto-analyzer (Technicon Systems, Inc.). Available phosphorus (P-Olsen) was extracted using a 0.5 M solution of NaHCO$_3$ at pH 8.5 and determined according to Olsen and Sommers [39] and concentrations of K, Na and Mg were determined after CaCl$_2$ extraction [40]. Total soil N and P content were determined by digestion with a mixture of H$_2$SO$_4$-Se and salicylic acid [41]. Total soil N and P content were determined by digestion with a mixture of H$_2$SO$_4$-Se and salicylic acid [41]. Total soil N and P content were determined by digestion with a mixture of H$_2$SO$_4$-Se and salicylic acid [41]. Total soil N and P content were determined by digestion with a mixture of H$_2$SO$_4$-Se and salicylic acid [41]. Total soil N and P content were determined by digestion with a mixture of H$_2$SO$_4$-Se and salicylic acid [41]. Total soil N and P content were determined by digestion with a mixture of H$_2$SO$_4$-Se and salicylic acid [41].

| Soil Proportion | Ergosterol (mg kg$^{-1}$) | Nematodes (per 100 g soil dw) |
|----------------|--------------------------|------------------------------|
|                | Total | Bacterial | Plant | Fungal | Omni/Carnivores |
| ORG None | Unmixed | 0.80 | 1502 | 805 | 533 | 74 | 89 |
| ORG E 1 | 1:5 | 0.83 | 1685 | 865 | 623 | 97 | 100 |
| ORG E 1 | 1:1 | 0.89 | 2053 | 966 | 803 | 142 | 121 |
| ORG E 2 | 1:5 | 0.78 | 1664 | 846 | 532 | 95 | 191 |
| ORG E 2 | 1:1 | 0.76 | 1989 | 930 | 529 | 137 | 393 |
| ORG M 1 | 1:5 | 1.15 | 1897 | 1114 | 545 | 100 | 138 |
| ORG M 1 | 1:1 | 1.87 | 2688 | 1734 | 569 | 151 | 235 |
| ORG M 2 | 1:5 | 1.16 | 2686 | 1661 | 740 | 167 | 139 |
| ORG M 2 | 1:1 | 1.88 | 5053 | 3373 | 1154 | 353 | 238 |
| ORG L 1 | 1:5 | 0.85 | 2318 | 1379 | 703 | 115 | 121 |
| ORG L 1 | 1:1 | 0.96 | 3951 | 2529 | 1042 | 195 | 185 |
| ORG L 2 | 1:5 | 2.00 | 2120 | 988 | 882 | 150 | 100 |
| ORG L 2 | 1:1 | 4.40 | 3357 | 1356 | 1578 | 301 | 123 |
| MIN None | Unmixed | 0.45 | 742 | 282 | 372 | 53 | 34 |
| MIN E 1 | 1:5 | 0.54 | 1052 | 430 | 489 | 79 | 54 |
| MIN E 1 | 1:1 | 0.72 | 1673 | 725 | 722 | 131 | 94 |
| MIN E 2 | 1:5 | 0.50 | 1031 | 411 | 398 | 77 | 145 |
| MIN E 2 | 1:1 | 0.59 | 1609 | 669 | 448 | 126 | 366 |
| MIN M 1 | 1:5 | 0.86 | 1264 | 679 | 411 | 82 | 92 |
| MIN M 1 | 1:1 | 1.69 | 2308 | 1473 | 488 | 140 | 207 |
| MIN M 2 | 1:5 | 0.87 | 2052 | 1226 | 606 | 149 | 93 |
| MIN M 2 | 1:1 | 1.70 | 4673 | 3112 | 1074 | 342 | 211 |
| MIN L 1 | 1:5 | 0.56 | 1685 | 944 | 569 | 97 | 75 |
| MIN L 1 | 1:1 | 0.79 | 3571 | 2268 | 962 | 185 | 157 |
| MIN L 2 | 1:5 | 1.71 | 1487 | 553 | 747 | 132 | 54 |
| MIN L 2 | 1:1 | 4.22 | 2976 | 1094 | 1497 | 290 | 95 |

The table above shows the biotic soil characteristics of the soils at the start of the experiment, for nematodes abundances are given for their total and per feeding group (bacterial, plant, fungal feeders, omni- and carnivores).

Table 5. Biotic soil characteristics of the soils at the start of the experiment, for nematodes abundances are given for their total and per feeding group (bacterial, plant, fungal feeders, omni- and carnivores).

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the soil mixtures with a moisture content of 20%(w:w). Seedlings were planted three weeks after their germination and harvested after ten weeks of growth. The plants were grown in a greenhouse under the same controlled conditions as the plant communities of the main experiment.

**Measurements**

**Plants.** After 4 months of growth in the main experiment and 2.5 months of growth in the additional experiment, shoots and roots of each plant species were collected, dried to constant weight at 70°C and weighed. The reported total biomass comprises shoot plus root biomass. From each pot of the main experiment, a 300 g soil subsample was collected for the analysis of soil abiotic and biotic characteristics.

**Abiotic soil characteristics.** Soil mineral content was determined using sieved (4 mm mesh) fresh soil of all 8 unmixed field soils before the experiment and of all 168 pots at the end of the main experiment. Mineral N was extracted from soil subsamples (10 g dry weight eq.) by shaking in 50 ml 1 M KCl for 2 h, and filtering through a Whatman filter. The concentrations of NH$_4$ and NO$_3$ in the filtrate were determined colorimetrically using Traacs 800 auto-analyzer (Technicon Systems, Inc.). Available phosphorus (P-Olsen) was extracted using a 0.5 M solution of NaHCO$_3$ at pH 8.5 and determined according to Olsen and Sommers [39] and concentrations of K, Na and Mg were determined after CaCl$_2$ extraction [40]. Total soil N and P content were determined by digestion with a mixture of H$_2$SO$_4$-Se and salicylic acid [41]. Soil organic matter (OM) content was determined via loss on ignition of dry soil burned at 430°C as a percentage of total weight. Soil %C and %N in oven-dry soil was determined using an elemental analyser (Eager EA1112, Interscience, Breda). Soil water content was determined gravimetrically from fresh and oven-dry (105°C) soil and pH of fresh soil was measured in 1:2.5 (dry weight) soilwater suspensions. Soil texture was determined using soil particle sizes distributions of freeze dried, sieved (1 mm mesh) soil, measured by laser diffraction with a Malvern 2000 particle size analyzer (Malvern Instruments Ltd, Malvern, UK). The proportion of mineral particles <2 μm were assigned to the clay fraction, particles of 2–50 μm to the loam and 60–1000 μm to the sand fraction.

**Biotic soil characteristics.** Ergosterol, as a measure of soil fungal biomass [42], was extracted from soil at the start and at the end of the main experiment and quantified by HPLC analysis by standard procedures [26]. Nematodes were extracted from 100 cm$^3$ of fresh soil by Oostenbrink elutriators [43], extracts were poured on a double cotton wool filter (Hygia milac filter, Hartmann BV, Nijmegen, the Netherlands), put on a tray with
100 ml water from which nematodes were collected after 24 hours incubation at 20°C and concentrated into 10 ml volume. All nematodes in 2 ml subsamples were examined using a reverse-light microscope (×100–400), counted and classified into feeding-groups according to Yeates et al. [44] as bacterial feeders, plant feeders (including plant associated nematodes), fungal feeders and omn/carnivores.

Data analysis

Plant evenness in the main experiment was calculated as Simpson's evenness index SEI which equals $1/\sum p_i^2 \times 1/S$, where $p_i$ represents the proportional contribution of shoot biomass of species $i$ to the total plant community shoot biomass and $S$ is the number of species in the community [45]. Soil characteristics of the 26 soil treatments at the start of the main experiment were based on the data of the unmixed soils and the proportion in which they were mixed. To test effects of receptor soil type and of donor soil origin and their interaction in the main and in the additional experiment two-way Analysis of Variance (ANOVA) was performed with type of arable receptor soil (organic or mineral), donor soil origin (one of the six grasslands or no addition) and receptor x-donor soil as fixed factors and block as a random factor. In the main experiment two-way ANOVA was applied separately for the dataset comprising the small (1:5) and large (1:1) additions of donor soil and for the dataset with soil of the main experiment as inoculum for sterilised soil. The effects of the proportion of added donor soil were tested on the dataset comprising all treatments with donor soil addition, but excluding the treatments with unmixed receptor soils, by means of ANOVA with donor soil, receptor soil, proportion of donor soil and their interactions as fixed and block as random factors. Differences between the treatments were tested using Tukey's posthoc tests (for unequal N in cases of unequal number of replicates) or LSD test (for A. dioica mass in the additional experiment). Homogeneity of variance was verified using Levene's test and biomass, SIEI values and ergosterol end concentrations were sqrt transformed to achieve homoscedasticity, ANOVAs were performed using STATISTICA (release 9.0, Statsoft, Inc.), Relations between plant species biomass and initial soil abiotic and biotic characteristics of the 26 soil treatments (24 mixtures and unmixed mineral and organic arable soil) in the main experiment were analysed by multivariate redundancy analysis (RDA) and Monte Carlo permutation tests (499 unrestricted permutations) using CA-NOCO, version 4.5 [46].

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Author Contributions

Conceived and designed the experiments: VC WHvdP GBDD. Performed the experiments: VC BeB GBDD. Analyzed the data: VC BeB GBDD. Contributed reagents/materials/analysis tools: WvdP. Wrote the paper: VC WHvdP GBDD.

References

1. Stevens CJ, Dice NB, Mountford JO, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. Science 303: 1876–1879.
2. de Bello F, Lavorel S, Gerhold P, Reier U, Parrel M (2010) A biodiversity monitoring framework for practical conservation of grasslands and shrublands. Biological Conservation 143: 9–17.
3. Grime JP (1987) Evidence for existence of 3 primary strategies in plants and its relevance to ecological and evolutionary theory. The American Naturalist 111: 1169–1194.
4. Marrs RH (1993) Soil fertility and nature conservation in Europe: Theoretical considerations and practical management solutions. Advances in Ecological Research 24: 241–300.
5. Walker KJ, Stevens PA, Stevens DP, Mountford JP, Manchester SJ, et al. (2004) The restoration and recreation of species-rich lowland grassland on formerly managed for intensive agriculture in the UK. Biological Conservation 119: 1–18.
6. Pywell RF, Bullock JM, Hopkins A, Walker KJ, Sparks TH, et al. (2002) Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. Journal of Applied Ecology 39: 294–309.
7. Pywell RF, Bullock JM, Tallon JB, Walker KJ, Warman EA, et al. (2007) Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints. Journal of Applied Ecology 44: 81–94.
8. Bakker JP, Berendse F (1998) Constraints in the restoration of ecological diversity in grassland and heathland communities. Trends in Ecology and Evolution 14: 63–68.
9. Olff H, Ritchie ME (1998) Effects of herbivores on grassland plant diversity. Trends in Ecology and Evolution 13: 261–265.
10. Blumenthal DM, Jordan NR, Ruselle MP (2003) Soil carbon addition controls weeds and facilitates prairie restoration. Ecological Applications 13: 605–615.
11. Eschen R, Mortimer SR, Lawson CS, Edwards AR, Brook AJ, et al. (2007) Carbon addition alters vegetation composition on ex-arable fields. Journal of Applied Ecology 44: 95–104.
12. Frouz J, Van Diggelen R, Pizl V, Stary J, Hanel L, et al. (2009) The effect of topsoil removal in restored heathland on soil fauna, topsoil microstructure, and fungal biomass development in a chronosequence of land abandonment. Soil Biology and Biochemistry 41: 2006–2013.
13. Lindborg R, Eriksson O (2004) Historical landscape connectivity affects present plant species diversity. Ecology 85: 1840–1845.
14. Ozinga WA, Romerermann C, Bekker RM, Priming A, Tamis WLM, et al. (2009) Dispersal failure contributes to plant losses in NW Europe. Ecology Letters 12: 66–74.
15. Fagan KC, Pywell RF, Bullock JM, Marrs RH (2008) Do restored calcareous grasslands on former arable fields resemble ancient targets? The effect of time, methods and environment on outcomes. Journal of Applied Ecology 45: 1293–1303.
16. Kardol P, Van der Wal A, Bezemér TM, de Boer W, Duys H, et al. (2008) Restoration of species-rich grasslands on ex-arable land: Seed addition outweighs soil fertility reduction. Biological Conservation 141: 2208–2217.
17. Kirsch K, Kimmer A, Donath TW, Rievan L, Holzel N (2010) Species introduction in restoration projects - Evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. Basic and Applied Ecology 11: 285–299.
18. Turner VT, Hawkins GV (2000) Embracing variability in the application of plant-soil interactions to the restoration of communities and ecosystems. Restoration Ecology 16: 713–729.
19. Harris J (2009) Soil microbial communities and restoration ecology: facilitators or followers? Science 325: 573–574.
20. Bever JD (2005) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytologist 157: 465–473.
21. De Deyn GB, Raaijmakers CE, Zoomer HR, Berg MP, de Ruiter PC, et al. (2003) Soil invertebrate fauna enhances grassland succession and diversity. Nature 422: 711–713.
22. Kardol P, Bezemér TM, van der Putten WH (2006) Temporal variation in plant-soil feedback controls succession. Ecology Letters 9: 1080–1088.
23. Parlevliet EJ, Bever JD (2011) Inoculation with a native soil community advances succession in a grassland restoration. Restoration Ecology (in press) doi: 10.1111/j.1526-100X.2010.00752.x.
24. Wardle DA, Bardgett RD, Klironomos JN, Setala H, van der Putten WH, et al. (2009) Ecological linkages between aboveground and belowground biota. Ecology 16: 713–729.
25. Kardol P, Bezemer TM, van der Wal A, van der Putten WH (2005) Successional trajectories of soil nematode and plant communities in a chronosequence of ex-arable lands. Biological Conservation 126: 317–327.
26. van der Wal A, van Veen JA, Smant W, Bousker HTS, Bloem J, et al. (2006) Fungal biomass development in a chronosequence of land abandonment. Soil Biology and Biochemistry 38: 51–60.
27. Piotrowski JS, Rilgic MC (2008) Succession of arbuscular mycorrhizal fungal patterns, causes, and considerations for organic agriculture. Advances in Agronomy 97: 111–130.
28. De Deyn GB, Raaijmakers CE, van der Putten WH (2004) Plant community development is affected by nutrients and soil biota. Journal of Ecology 92: 824–834.
29. Johnson NC, Rowland DL, Corkidi L, Egerton-Warburton LM, Allen EB (2003) Nitrogen enrichment alters mycorrhizal allocation at five mesic to semiarid grasslands. Ecology 84: 1905–1908.
30. Collins CD, Foster BL (2009) Community-level consequences of mycorrhizae depend on phosphorus availability. Ecology 90: 2567–2576.

31. Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, et al. (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecology Letters 13: 394–407.

32. Suding KN, Gross KL, Houseman GR (2004) Alternative states and positive feedbacks in restoration ecology. Trends in Ecology and Evolution 19: 46–53.

33. Kardol P, Wardle DA (2010) How understanding aboveground-belowground linkages can assist restoration ecology. Trends in Ecology and Evolution 25: 670–679.

34. Kardol P, Bezemer TM, van der Putten WH (2009) Soil organism and plant introductions in restoration of species-rich grassland communities. Restoration Ecology 17: 238–269.

35. Reynolds HL, Haubensak KA (2009) Soil fertility, heterogeneity, and microbes: towards an integrated understanding of grassland structure and dynamics. Applied Vegetation Science 12: 33–44.

36. Walters DR, Bingham IJ (2007) Influence of nutrition on disease development caused by fungal pathogens: implications for plant disease control. Annals of Applied Biology 151: 307–324.

37. Hol WHG, de Boer W, Teunenhuizen AJ, Meyer KM, Schneider JHM, et al. (2010) Reduction of rare soil microbes modifies plant-herbivore interactions. Ecology Letters 13: 292–301.

38. Schaminée JHJ, Stortelder AHF, Weeda EJ (1996) De vegetatie van Nederland. Deel 3. Plantengemeenschappen van graslanden, zomen en droge heiden. Opulus Press, Uppala.

39. Olsen SR, Sommers LE (1982) Phosphorous. In: Al, editor. Methods of soil analysis. Part 2. Chemical and microbiological properties. Agronomy Monograph 9, ASSA and SSSA, Madison. pp 403–430.

40. Houba VJG, van der Lee JJ, Walinga I, Novozamsky I (1985) Soil analysis. Part 2: Procedures. Wageningen University, Wageningen.

41. Novozamsky I, Houba VJG, Temminghoff E, van der Lee JJ (1984) Determination of total N and total P in a single soil digest. Netherlands Journal of Agricultural Sciences 32: 322–324.

42. Baath E (2001) Estimation of fungal growth rates in soil using C-14 acetate incorporation into ergosterol. Soil Biology and Biochemistry 33: 2011–2018.

43. Oostenbrink M (1960) Estimating nematode populations by some selected methods. In: Sasser NJ, Jenkis WR, eds. Nematology University of North Carolina Press, Chapel Hill. pp 85–102.

44. Yeates GW, Bongers T, de Goede RGM, Freckman DW, Georgiev SS (1993) Feeding habits in soil nematode families and genera – an outline for soil ecologists. Journal of Nematology 25: 315–331.

45. Simpson EH (1949) Measurement of diversity. Nature 163: 688.

46. Ter Braak CJF, Smilauer P (1998–2002) Canoco for Windows 4.5. Biometris, Wageningen-UR.