Nematode-plant interactions in grasslands under restoration management

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Abstract

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Plant-feeding nematodes may have a considerable impact on the rate and direction of plant succession. In this thesis the interactions between plants and plant-feeding nematodes in grasslands under restoration management were studied. In these grasslands, a management of ceasing fertiliser application and annual hay-making resulted in a succession of high- to low-production plant communities. It was hypothesised that a reduced nutrient availability and the development of species-specific nematode communities under plant species will increase the sensitivity of plant species to nematode herbivory. This may result in the replacement of such plant species by plant species that are better adapted to nutrient-poor conditions. The reduction in nutrient supplies resulted in a gradual succession of plants and plant-feeding nematodes. Alterations in the species composition of the plant community, but particularly qualitative changes within each plant species after the cessation of fertiliser application affected the plant-feeding nematode succession. Indications were found that the nematode numbers were positively related to the root nitrogen concentrations. Estimations of nematode consumption in the field indicated that in local hotspots nematodes may have a considerable impact on plant productivity. In experimental studies, however, the effects of nematodes on plant productivity were in general small. Some evidence was found that plant-feeding nematodes can affect the competition between an early- and late-successional plant species in favour of the latter, but I did not find experimental evidence that plants under nutrient-poor growth conditions were more sensitive to nematode herbivory. Neither did I find clear-cut evidence for species-specific suppression of plant species by nematodes. It was suggested, therefore, that in addition to progressing nutrient stress, plant species-specific differences in tolerance to plant-feeding nematodes, rather than host specificity of nematodes, may determine the plant species replacement during reversed succession in grasslands. It is concluded that plant-feeding nematodes are potentially an important biotic factor in the succession of plant communities, but their impact on the succession in grasslands under restoration management has yet to be further elucidated. So far the results suggest that the succession of the plant-feeding nematode community is probably more affected by changes in the plant community than the other way round.

Keywords: competition, fertilisation, food quality, grassland, herbivory, nitrogen, nutrients, plant-feeding nematodes, productivity, restoration management, succession, synergism, vegetation

Contents

1. General introduction

HERBIVORY AND SUCCESSION

Succession refers to the colonisation and development of ecological communities in new primary habitats (primary succession) or to temporal changes in ecological communities following a disturbance (secondary succession). The process of succession has intrigued many ecologists during the last century and incited them to study the underlying mechanisms (Clements, 1916; Olson, 1958; Connell and Slatyer, 1977; Tilman, 1985). The importance of the various suggested mechanisms, however, strongly depends on the type of disturbance, the uniqueness of species and habitats, as well as various historical factors. Tilman (1985), therefore, proposed a resource-ratio hypothesis that was meant to explain general patterns observed in different kinds of succession. According to this hypothesis, succession results from a gradient through time in the relative availabilities of limiting resources. When each species is considered a superior competitor for a particular combination of limiting resources, the species composition will change whenever the relative availability of limiting resources changes.

Tilman (1985) developed his model for plant species, but did not include in it herbivore-related processes. Connell and Slatyer (1977) suggested that, in addition to the competitive interactions between plants, interactions with herbivores and pathogens are of critical importance to the course of succession. In the last two decades a considerable amount of evidence of herbivore/pathogen-affected plant succession has been found (Brown, 1985; Edwards and Gillman, 1987; Gibson et al., 1987; Brown and Gange, 1992; Van der Putten et al., 1993; Bach, 1994; Jefferies et al., 1994; Mortimer et al., 1999; Van der Putten, in press). Following up on Tilman's resource-ratio hypothesis, herbivores and pathogens may affect plant competition by changing the relativeavailabilities of resources. Firstly, herbivores and pathogens can affect plant growth, thereby reducing the resource uptake capacity of damaged plants. Secondly, they can change the rates of nutrient supply by affecting the nutrient

turnover from plant to soil. Moreover, herbivores can stimulate the colonisation of new species by creating gaps in plant communities and may increase plant dispersal by transporting plant seeds (Edwards and Gillman, 1987).

PLANT-FEEDING NEMATODES AND SUCCESSION

Nematodes have frequently been studied in successional series of different habitats (Wasilewska, 1970; De Goede et al., 1993a,b; Wasilewska, 1994; Hánel, 1995; Armendáriz et al., 1996; Brzeski, 1996; Brinkman et al., 1998), but just recently their possible importance as a biotic factor in successional processes has been recognised (Mortimer et al., 1999). In sand dunes plant-feeding nematodes were supposed to be involved in the degeneration of dominant plant species, such as *Hippophaë rhamnoides* (Sea buckthorn) and *Ammophila arenaria* (Marram grass) (Oremus and Otten, 1981; Maas et al., 1983; Van der Putten et al. 1988; Van der Putten et al., 1990; De Rooij-van der Goes, 1995; Zoon, 1995). Furthermore, plant-feeding nematodes might have contributed to small-scale shifts in the vegetation composition of grasslands (Blomqvist et al., 2000; Olff et al., 2000). Such effects of plant-feeding nematodes on vegetation dynamics might have been caused by the direct inhibition of plant growth as a result of their feeding activity. It is generally supposed, however, that nematodes might indirectly affect plant growth by increasing the susceptibility of plants to pathogenic micro-organisms (De Rooij-Van der Goes, 1995). As components of pathogen communities, therefore, plant-feeding nematodes may contribute to vegetation succession (Van der Putten et al., 1993; Van der Putten and Peters, 1997; Van der Putten and Van der Stoel, 1998).

NEMATODE HERBIVORY IN GRASSLANDS

Belowground herbivores are considered to be major consumers in (semi)-natural grasslands. Scott et al. (1979) calculated that only 2-7% of the total primary production in grasslands is consumed aboveground, whereas this is 7-26% belowground. Furthermore, the amount of plant biomass that is wasted by herbivores during feeding can be considerable. In total, the proportion of primary production that is eaten and wasted by herbivores was calculated to be 3-10% aboveground and 13-41% belowground. Plant-feeding nematodes were found to be the major consumers in grasslands. They were responsible for 46 to 67% of the total plant consumption (Scott et al., 1979). The importance of plant-feeding nematodes in grasslands is

supported by field studies in which the application of nematicides resulted in increased grassland yields of 25 to 59% (Smolik, 1977; Stanton et al., 1981; Ingham et al., 1986b; Ingham and Detling, 1990).

OBJECTIVES OF THE THESIS

In former species-rich grasslands in the Drentse A nature reserve in the northern part of the Netherlands (53°N, 6°42'E), long-term application of fertilisers had resulted in high-production grassland communities with a low plant biodiversity. In order to restore the former species-rich plant communities, the Dutch State Forestry Commission applies a management strategy of ceasing the application of fertilisers while nutrients are removed by haymaking. Such management resulted in a decrease of the soil nutrient pools and reduced rates of both nitrogen mineralisation and nitrification (Olff et al., 1994; Stienstra et al., 1994). Consequently, the vegetation first became limited by nitrogen and potassium and later also by phosphorus, resulting in a reversed succession in which fast-growing, light-competing plant species were replaced by slow-growing nutrient-competitors (Table 1.1; Olff, 1992a; Olff and Pegtel, 1994). Furthermore, the total plant production decreased with increased time of non-fertilisation (Olff and Bakker, 1991).

Table 1.1. Dominant plant species in four grasslands of the Drentse A nature reserve, which had not been fertilised for 6, 10, 23 and 28 years. The plant species are presented in decreasing order of dominance.

Years not fertilised			
6	10	23	28
Lolium perenne L.	*Holcus lanatus* L.	*Festuca rubra* L.	*Festuca rubra* L.
Holcus lanatus L.	*Agrostis stolonifera* L.	*Agrostis capillaris* L.	*Agrostis capillaris* L.
Agrostis stolonifera L.	*Anthriscus sylvestris*	*Holcus lanatus* L.	*Anthoxanthum odoratum* L.
Ranunculus repens L.	(L.) Hoffm.		Mosses

The aim of the research presented in this thesis is to investigate the effects of plant-feeding nematodes on the vegetation succession in the grasslands of the Drentse A nature reserve. Belowground herbivores, in particular plant-feeding nematodes, are supposed to be an important biotic factor affecting the vegetation development in these grasslands (Brussaard et al., 1996). On the other hand the productivity and composition of the plant community may

affect the abundance and composition of the plant-feeding nematode community (Yeates, 1987).

The main hypothesis of the thesis is that root herbivory by plant-feeding nematodes affects the competition between early- and late-successional plant species in favour of the latter, leading to an acceleration of plant succession. Such suppression of early-successional plant species by late-successional species can be caused by two mechanisms. Firstly, early-successional plant species may be more sensitive to nutrient-poor conditions than late-successional species. An increased stress due to lower nutrient availabilities, is assumed to cause a higher sensitivity to nematode infestation (White, 1984) and, subsequently, lower competitive abilities of the early-successional species. Secondly, species-specific nematode communities may develop under the early-successional species which may affect the growth of these plant species, but not that of their successors (Van der Putten et al., 1993).

On the other hand successional changes in the plant community will affect the composition of the plant-feeding nematode community. It is hypothesised that a reduction in nutrient availability for the plant will result in a lower food quantity and quality for plant-feeding nematodes. Subsequently, the abundance of plant-feeding nematodes will decrease and nematode species with relatively low nutrient-use efficiencies will be replaced by species with higher nutrient-use efficiencies.

These hypotheses are schematically represented in Fig 1.1. In fertilised nutrient-rich habitats, the dominant plant species are supposed to have high growth rates, turnover rates and nutritional qualities and low nutrient-use efficiencies. The high nutritional quality of these plant species results in a high density of plant-feeding nematodes that are characterised by high growth and turnover rates, and low nutrient-use efficiencies. Furthermore, nematode communities may develop in the soil that are specific for the plant species present. A reduction in the availability of nutrients will result in higher nutrient stress for the early-successional plant species. Subsequently, the sensitivity of these plants to nematode herbivory will increase which may result in an accelerated replacement of the early-successional plant species by late-successional plant species which are less susceptible to the (species-specific) nematodes and are better adapted to more nutrient-poor conditions. Plant-feeding nematodes may, therefore, speed up succession by affecting the early-successional plant species relatively more than their successors (Schowalter, 1981).

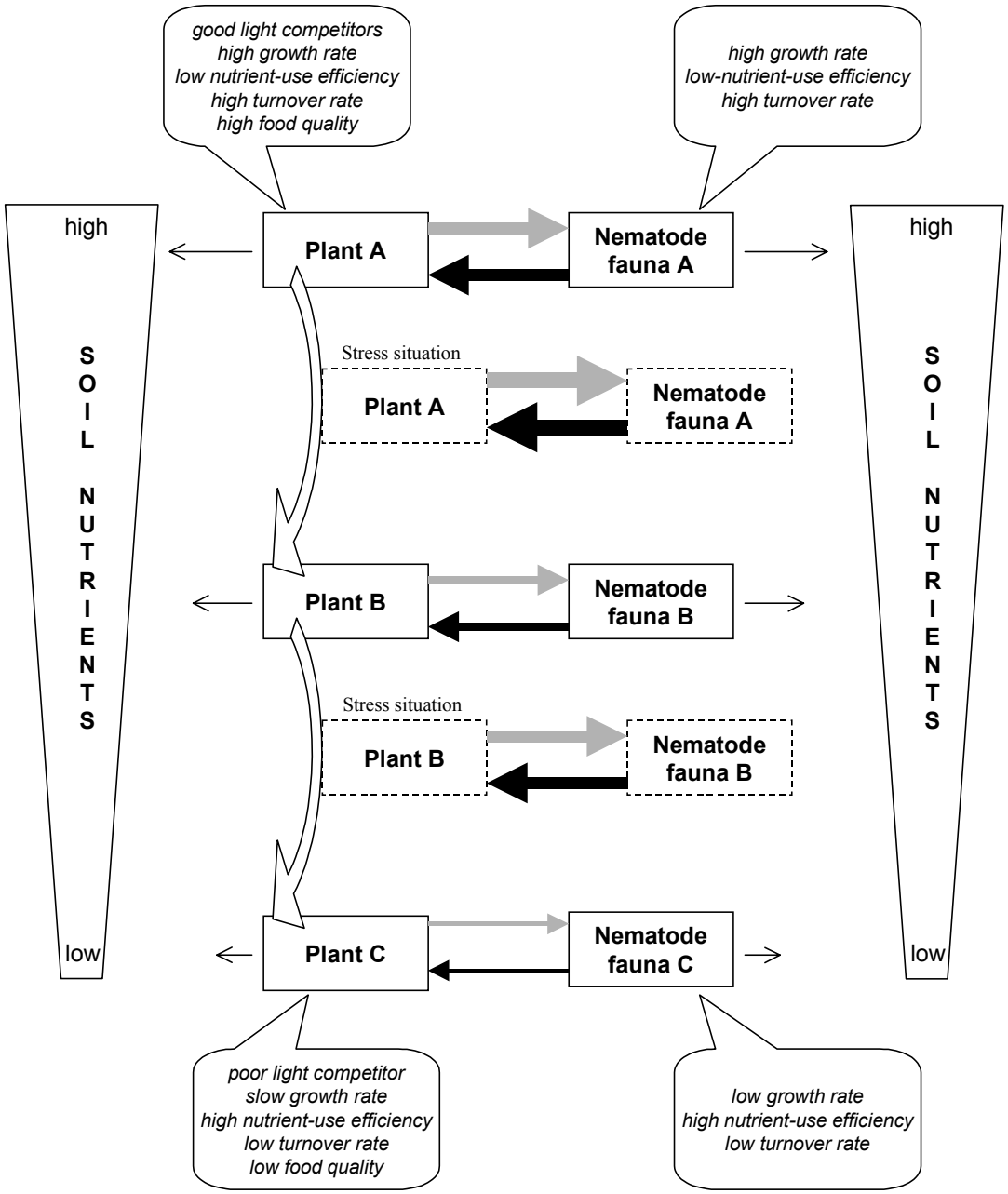


Figure 1.1. Schematic presentation of the effects of nutrient impoverishment in grasslands after cessation of fertiliser application on the interactions between plants and plant-feeding nematodes. Grey arrows represent the positive effects of plants on the numbers of plant-feeding nematodes. Black arrows represents the negative effects of nematode herbivory on plant growth. The thickness of the grey and black arrows gives an indication of their impact. White arrows represent the replacement of plant species. The small arrows represent the turnover rate of nutrients to the soil by plants and nematodes. The length of these arrows gives an indication of their impact. Species characteristics of plants and nematodes of nutrient-rich and nutrient-poor habitats are given in the call-outs.

The succeeding plant species, which are better adapted to the more nutrient-poor conditions, can be characterised by slower growth and turnover rates, and higher nutrient-use efficiencies. Slow-growing plant species generally have a lower nutritional quality (Coley, 1988; Southwood et al., 1986), which may result from a higher physical and chemical defence (Edwards-Jones and Brown, 1993) or a lower N-content (Mattson, 1980; Lambers and Poorter, 1992). Consequently, the abundance of plant-feeding nematodes will decrease with time of non-fertilisation and nematode species with low nutrient-use efficiencies will be replaced by species with higher nutrient-use efficiencies. Furthermore, new species-specific nematode communities will develop under these late-successional plant species that also contribute to a progressing succession.

Fast-growing plant species of nutrient-rich habitats generally have higher turnover rates of biomass to the soil than slow-growing species of nutrient-poor habitats (Van der Krift, 2000). Consequently, the absolute contribution of plants to the soil nutrient pool decreases with the time of succession. Plant-feeding nematodes can increase the nutrient transfer from plant to soil by root consumption and by enhancing root exudation. Particularly under nutrient-rich conditions the contribution of nematodes to nutrient turnover is assumed to be high due to high nematode numbers and low nutrient-use efficiencies. Under nutrient-poor conditions, however, plant-feeding nematode communities are expected to be less abundant and to have higher nutrient-use efficiencies. It is hypothesised, therefore, that the absolute contribution of nematode herbivory to nutrient turnover will decrease with time of non-fertilisation.

OUTLINE OF THE THESIS

A survey on the occurrence of plant-feeding nematodes was carried out in four grasslands that differed in their time of non-fertilisation (chapter 2). The effects of time of non-fertilisation, plant species composition, season and environmental conditions on the composition of the plant-feeding nematode communities were investigated by means of multivariate analyses. The hypothesis was tested that nutrient impoverishment of the grasslands would result in a decrease in the nematode density and biomass and a shift in the nematode community structure towards species that are better adapted to nutrient-poor conditions. Furthermore, the seasonal changes and vertical distribution of the dominant plant-feeding nematode taxa in these grasslands and their relationships with (a)biotic conditions were investigated (chapter 3).

On the basis of these results, the yearly biomass consumption of plant-feeding nematodes and their effects on the carbon and nitrogen turnover of plants to the soil in the studied grasslands were estimated (chapter 4).

The next aspect investigated was the effect of root herbivory by nematodes on plant growth in relation to soil nutrient availability. In a greenhouse experiment, an early-successional plant species, *Lolium perenne*, and a late-successional plant species, *Festuca rubra*, were grown in sterilised soil at low and high nutrient supply rates, with or without nematodes from an early- and late successional grassland (chapter 5).

In a second greenhouse experiment, the effects of plant-feeding nematodes on competition between an early- and a late-successional plant species were investigated (chapter 6). In this experiment, *Holcus lanatus* and *Anthoxanthum odoratum* were grown on soil of an early-successional grassland that was either treated or untreated with nematicide. The plant species were grown in mono- and mixed cultures according to a De Wit replacement series.

To determine the effects of root herbivory by nematodes on the net primary production of grasslands under natural field conditions, a field experiment was carried out at different stages of succession (chapter 7). In four grasslands, therefore, plots of 1×1 m were treated with nematicide or remained untreated. Furthermore, these treatments were carried out with or without the application of fertilisers and lime to examine the interactive effects of nematodes, nutrient availability and soil pH. At the end of the growing season the standing biomass and species composition of the different treatments were determined. Moreover, four different plant species were planted within the experimental plots to investigate the treatment effects on single plant species.

Host specificity of soil-borne pathogens is supposed to be an important factor affecting vegetation dynamics. In a greenhouse experiment the specificity of the dominant plant-feeding nematode species for dominant plant species of different stages of succession was determined (chapter 8).

The results of the preceding chapters are synthesised and discussed in the general discussion (chapter 9).

VERSCHOOR, B.C., DE GOEDE, R.G.M., DE VRIES, F.W., BRUSSAARD, L. (2001) Changes in the composition of the plant-feeding nematode community in grasslands after cessation of fertiliser application. *Applied Soil Ecology* 17: 1-17.

2. Changes in the composition of the plant-feeding nematode community in grasslands after cessation of fertiliser application

Abstract The community structure and abundance of plant-feeding nematodes were studied in four grasslands, which had not been fertilised for 6, 10, 23, and 28 years, respectively. We hypothesised that nutritional impoverishment of the grasslands would result in a decrease of the nematode density and biomass, and a shift in the nematode community structure towards species that are better adapted to nutrient-poor conditions. Furthermore, we expected that plant-specific nematode communities are present in the rhizosphere of different dominant plant species. Multivariate analyses showed gradual changes in the nematode fauna after the cessation of fertiliser application. Particularly, during the first ten years of succession the density and species number of large endo- and ectoparasites strongly decreased resulting in a lower diversity of nematode genera. Root biomass, plant species, and season had a minor effect on the nematode numbers and community composition. However, within the rhizosphere of single plant species clear community changes were observed with time of non-fertilisation, indicating that qualitative changes within a plant species rather than qualitative differences between plant species affected the nematode community. Most likely, a lower nutritional quality of the nematode food source, due to decreased nutrient concentrations and increased levels of defensive compounds in plant tissues under nutrient-poor conditions, was the major factor affecting the plant-feeding nematode community. Average nematode body size, which is constrained by the absolute amount of food necessary for growth and maintenance, decreased at a lower nutrient availability. However, the results suggest that evolutionary adaptation of feeding strategies that reduces the energetic costs of feeding and movement, such as endoparasitism, a longer stylet, and the modification of feeding cells, enabled certain nematode species to support a relatively large body size at a low nutrient availability.

Introduction

Recent restrictions in agricultural production in Europe and the demands of nature conservationists, have resulted in an increase of abandoned arable fields and grasslands under restoration management. The nutrient availability in these areas is usually high due to long-term excessive application of fertiliser. Such areas are characterised by species-poor plant communities that are dominated by a few fast-growing, light-competing plant species. Reduction of soil fertility is one of the measures to restore the former species-rich plant communities. In grasslands along small streams in the northern part of the Netherlands this was attained by haymaking after the cessation of fertiliser application (Bakker, 1989; Olff and Bakker, 1991). As a result a low productive, nutrient-limited plant community developed consisting of slow-growing species.

Altered nutrient levels in the soil affect plant-feeding nematode communities (Smolik and Dodd, 1983; Dmowska and Ilieva, 1995; Todd, 1996; Yeates and King, 1997; Neher and Olson, 1999). Increased soil nutrient availability positively affects plant productivity and nutritional quality of plant tissues, and altered the plant composition of the vegetation. Generally, the density of nematodes increases with plant productivity in grasslands (Yeates, 1979; 1987). Furthermore, the nematode density is positively related to the nutritional quality of plants in terms of tissue-nutrient concentrations (Yeates, 1987; Todd, 1996).

Generally, nitrogen is a limiting factor for many herbivores (Mattson, 1980). The efficiency with which a consumer can convert ingested food into its own biomass is linearly related to the food N concentration. This implies that on a low-N diet organisms have to consume a substantially larger amount of food to get a sufficient amount of N for growth and maintenance of their body than on a high-N diet. Herbivores have evolved several adaptations to low N supplies such as (*a*) increased consumption rates, (*b*) prolonged periods of feeding, digestion, and development, (*c*) symbiotic relationships with microbes, (*d*) occasional carnivory, (*e*) switching among plant parts and plant species, (*f*) regulation of plant chemistry, and (*g*) evolution of larger body size (Mattson, 1980). However, Atkinson (1985) demonstrated that for plant-feeding nematodes the body size is first of all constrained by the absolute amount of food that a nematode can acquire per day. Therefore, feeding strategies that save energetic costs of feeding and movement could be a useful adaptation of plant-feeding nematodes to support a relatively large body size at a low food quality. Endoparasitism could be an efficient way to save energy, particularly for endoparasitic species which are able to modify plant cells into specialised feeding cells (syncytia) that

provide a continuous supply of nutrients to the nematode (Ferris, 1982). Furthermore, differentiation in the size of the stylet can be a useful adaptation. Like endoparasitism, a longer stylet provides nematodes with the opportunity to exploit new food resources (Yeates, 1986) and to feed for prolonged periods on the same feeding site.

We hypothesise that the number and biomass of plant-feeding nematodes decrease with increased period of non-fertilisation and that the nematode community will develop towards species that are better adapted to low nutrient conditions, such as endoparasites, species with longer stylets, and species with a smaller body size. Furthermore, we expect that host-specific plant-feeding nematode communities will be present in the rhizosphere of dominant plant species. This paper presents the results of a nematode survey that was carried out during a sixteen-month period in four grasslands that differ in time of non-fertilisation. The composition of species and feeding groups of the plant-feeding nematode community is described in relation to successional changes in the vegetation. Interactions between nematodes and specific dominant plant species were investigated on the assumption that the comparison of the four fields represents a comparison of temporal stages during secondary succession. Effects of time after cessation of fertiliser, plant species, root biomass, abiotic environmental conditions, and season (time of the year) were explored by means of redundancy analyses.

Materials and Methods

Study Area

The study was done in grasslands of the Drentse A nature reserve in the northern part of the Netherlands (53°N, 6°42'E). Along a small stream, the Anloërdiepje, four grasslands were selected which had not been fertilised for 6 (field O), 10 (field B), 23 (field C) and 28 (field K) years to represent different stages of secondary succession. Before fertilisation ceased, all fields were managed according to standard agricultural practice typical for permanent grassland. This included several combinations of haymaking, grazing by cows and fertilisation (100-250 kg N ha^{-1}, with sufficient P and K) (Olff et al., 1994). In field O grazing by cows was not stopped immediately, but continued for 5 years after cessation of fertiliser application. All grasslands were mown in July-August each year.

Long-term studies in grasslands in the study area have demonstrated that cessation of fertilisation resulted in a decrease of the soil C, N and P pools. Simultaneously, N mineralisation and nitrification decreased (Olff et al., 1994; Stienstra et al., 1994). Consequently, the vegetation first became limited by N and K and later also by P, resulting in a replacement of fast-growing light-competing plant species by slow-growing nutrient-competitors (Olff, 1992a; Olff and Pegtel, 1994). Furthermore, the total plant production decreased with increased time of non-fertilisation (Olff and Bakker, 1991).

In each study site a topographic gradient was present ranging from the lower, wetter parts near the stream to the sandy plateau along the valley. The nematode survey was conducted on the highest and driest parts of the fields near the edge of the plateau. In this part of the gradient the vegetation of field O was dominated by *Lolium perenne* L., *Holcus lanatus* L., *Agrostis stolonifera* L., and *Ranunculus repens* L., in field B by *H. lanatus*, *A. stolonifera*, and *Anthriscus sylvestris* (L.) Hoffm., in field C by *Festuca rubra* L., *H. lanatus*, and *Agrostis capillaris* L., and in field K by *F. rubra, A. capillaris, Anthoxanthum odoratum* L., mosses, and to a lesser extend *H. lanatus*.

The soil type in each field is loamy sand with a clay+silt content of 20-23% in all fields (table 2.1). The bulk density of the soil in field C was somewhat lower than in the other fields. With time of non-fertilisation the pH decreased, whereas the total amount of C, the organic C%, and the C:N ratio increased. The total amount of N was lowest in field B and highest in field C.

Table 2.1. Soil characteristics (mean values of three replicates) of the four sites studied at 0-10 cm depth [1].

	field O		field B		field C		field K	
years unfertilized	6		10		23		28	
soil bulk density (g/cm^3)	1.21	a	1.12	ab	1.03	b	1.17	a
pH (H_2O)	4.87	a	3.89	b	4.00	b	3.96	b
clay+silt%	23.0		21.8		19.9		21.9	
C-total (g/cm^3)	0.53	b	0.41	c	0.77	a	0.79	a
N-total (g/cm^3)	0.028	ab	0.021	c	0.034	a	0.026	bc
C:N ratio	19.3	b	24.4	b	23.5	b	35.0	a
organic C%	3.89	bc	3.60	c	5.63	a	5.31	ab

[1] C. van Griethuysen (unpublished data).
Values with the same letter were not significantly different (P<0.05).

SAMPLING

From September 1995 – December 1996 soil samples were collected from 10 × 15 m plots, one in each field. There were nine sampling events (01-09-95, 13-10-95, 30-11-95, 29-02-96, 17-04-96, 13-06-96, 7-08-96, 3-10-96, 27-11-96). Each plot was divided into one hundred quadrats of 1.5 m^2 each. Each soil sample was taken in one of the quadrats that was chosen randomly and had not been sampled before. Samples were taken with a split core sampler (diameter 47 mm) in the 0-10 cm mineral soil under the dominant plant species of a field, *i.e.* under *L. perenne* and *H. lanatus* in field O, *H. lanatus* in field B, *H. lanatus* and *F. rubra* in field C and *F. rubra*, *A. odoratum* and *H. lanatus* in field K. Five replicate samples were collected of each plant species per sampling event. In field K *H. lanatus* was sampled only in September-November 1995 and October 1996, because this species had declined dramatically in 1996. In February 1996 most of the soil was frozen which obstructed the sampling. Therefore in February, only a limited number of samples could be taken in field O, C, and K (and none in field B). A preliminary study had shown that the large nematode species *Longidorus elongatus* occurred in field O only. Therefore, in field O an additional soil sample was collected on every sampling occasion from the same quadrat to be used for the extraction of *L. elongatus*. All soil samples were transported intact and kept for not more than 10 days at 4°C before extraction.

EXTRACTION, COUNTING AND IDENTIFICATION

All soil samples were weighed and soaked in a small amount of water prior to extraction. Nematodes were extracted from the soil suspension using a modified Oostenbrink elutriator (Oostenbrink, 1960) and incubated 48 hours on a double cottonwool filter (Hygia milac filter). The remaining plant roots in the top sieve of the apparatus were collected, cleaned of soil particles, superficially dried with tissue paper and weighed to assess fresh biomass. The roots were then cut into pieces of about 5 mm long and macerated in a blender for 10 seconds. Endoparasitic nematode species were extracted from the macerated roots by means of the modified centrifuge flotation method of Coolen and d'Herde (1972). *Longidorus* nematodes were extracted from the additional soil cores using a modified Oostenbrink elutriator with a continuous water up-flow of 1000 ml per minute. Instead of using a cottonwool filter, the *Longidorus* suspension was poured on a 175 µm sieve and incubated in an extraction dish for 48 hours.

After extraction, a subsample of the nematode suspension was counted under a low magnification inverted microscope. Complete *Longidorus* samples were counted. Afterwards the nematodes were heat-killed and fixed in 4% formalin. The species composition of the plant-feeding nematode community of each soil sample was determined under high magnification (×400-1000) with a compound microscope. Nematodes collected from the root samples were identified in water suspensions under an inverted microscope (×100-400), because the presence of root fragments hampered the preparation of slides. About 200-300 specimens were examined in each soil or root sample for determination. The classification to feeding groups followed Yeates et al. (1993). Besides plant-feeders, the fungal-feeding nematodes were also identified. These fungal feeders might affect the productivity of arbuscular mycorrhiza (AM), which we regard as an extension of the plant root system.

BIOMASS CALCULATION

Nematode biomass was calculated with the formula of Andrássy (1956): $G(\mu g) = (W^2 \times L)/(16 \times 10^5)$. Adult body length L of each species was taken from Verschoor and de Goede (2000). Body width W was calculated from the ratio between L and the mean nematode L/W quotient (derived from Bongers, 1988). Biomass was calculated separately for juveniles and adults. The mean juvenile length was assumed to be half the mean adult length (Verschoor and de Goede, 2000).

ENVIRONMENTAL CONDITIONS

From March-December 1996 soil moisture content and soil temperature in each field were measured at 10-15 cm depth. Because such data were missing for the first six months of sampling, the soil moisture content was estimated from the differences between the soil fresh weight at sampling and soil fresh weight of the samples taken in August 1996 when the soil was very dry. These estimates were found to be highly correlated with the actual moisture content ($r^2 = 0.85$, $n = 20$; based on data collected from March-December 1996). Daily air temperature and precipitation data were derived from the local weather station in Eelde. The air temperature data were highly correlated to our measured soil temperatures ($r^2 = 0.98$, $n = 60$; based on data collected from March-December 1996). For statistical analyses the average daily air temperature and precipitation over the 21 days before sampling were used.

STATISTICS

Ordination analyses (computer package CANOCO 4 (Ter Braak and Smilauer, 1998)) were used to describe changes in the nematode population structure and to relate these changes to environmental factors (for detailed description of ordination methods see Ter Braak (1994) and Jongman et al. (1995)). A detrended correspondence analysis (DCA by segments) resulted in a gradient of <3 SD long, indicating linear and not unimodal relationships between the species and sample scores (Ter Braak and Smilauer, 1998). Therefore, differences in species composition of the samples were analysed with principal component analysis (PCA) which is a linear method of ordination. The relationship between species abundance and environmental conditions was investigated using a redundancy analysis (RDA) in which the axes (*i.e.* the site scores) are constrained to be a linear combination of a set of explanatory variables. All analyses were based on the absolute numbers of nematodes per 100 cm^3 of soil after ln(x+1) transformation. For some endoparasitic nematode taxa, i.e. *Pratylenchus*, *Subanguina* and *Heterodera* that were frequently found in the root samples (>10% of the total population), the numbers of nematodes found in the roots and in the soil were lumped preceding the analyses. The explanatory variables used were mean soil moisture content, mean air temperature, mean precipitation, root biomass, field (field O, B, C and K), plant species (*L. perenne*, *H. lanatus*, *F. rubra* and *A. odoratum*), and sampling date. Field, sampling date, and plant species were included as qualitative nominal variables. For the contribution of each explanatory variable to the total explained variance in the ordination analysis was calculated. Furthermore, by defining certain variables as covariables it was also possible to calculate the shared variance between two or more variables (Ter Braak and Smilauer, 1998). By such a decomposition of the variance it is possible to separate direct and indirect effects of the explanatory variables (Pyšek and Lepš, 1991). For these calculations we simplified our model by grouping the explanatory variables into five classes: field, plant species, root biomass, sampling date and abiotic factors. The decomposition of the variance might be complicated when interactive effects exist between variables. However, this appeared not to be a major problem for the present dataset.

To test species-specific differences in the composition of the nematode communities between the rhizospheres of different plant species, a Multivariate Analysis of Variance (MANOVA), which is more powerful than a univariate analysis (Stevens, 1996) was performed. Subsequently, Least Significant Difference tests (LSD's) were performed to test differences in nematode densities on a species level. Furthermore, nematode biomass and

diversity of genera between plant species and fields were tested by LSD. All data were ln(x+1) transformed before analysis to meet the rules of homogeneity of variance and normal distribution.

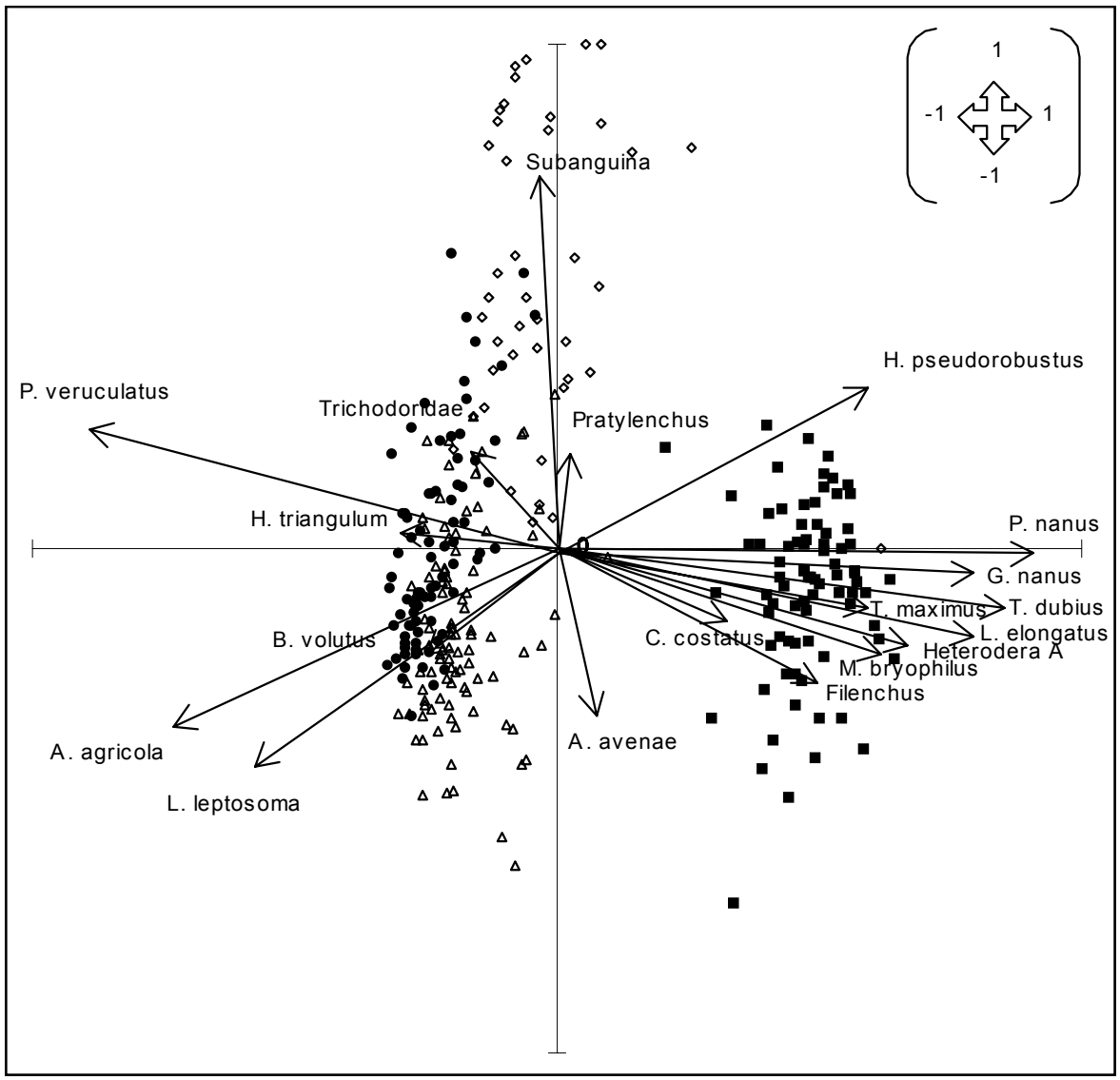


Figure 2.1. Correlation biplot of species and samples (axes 1 and 2) based on a PCA of the nematode densities of the four grasslands differing in time after cessation of fertiliser application (6 years, squares; 10 years, diamonds; 23 years, circles; and 28 years, triangles). The eigenvalues of the first four axes are 0.46, 0.08, 0.07, and 0.05, respectively.

Results

The first axis of the PCA explained 46% of the variance in the species data, whereas the second axis explained only 8% (Fig. 2.1). The ordering of the sites along the first two axes represented the fertilisation history of the fields. The samples of field O, in which fertilisation had been stopped 6 years, were characterised by a high diversity, abundance and biomass of plant-feeding nematodes (Table 2.2a/b). They were positioned in the right half of the ordination diagram and were highly dissimilar from the samples of the other fields (fields B, C, K). This was mainly the result of the presence of some nematode taxa that were more or less restricted to field O. Characteristic species of field O were the sedentary endoparasite *Heterodera* spec A, and the ectoparasites *Longidorus elongatus, Geocenamus nanus*, *Tylenchorhynchus dubius*, *Tylenchorhynchus maximus*, *Paratylenchus nanus* and *Malenchus bryophilus*. Some of these species were also found in the fields with a longer history of non-fertilisation, but then their abundance was significantly lower. Other species such as the semi-endoparasite *Helicotylenchus pseudorobustus* and the fungal-feeding *Aphelenchus avenae* were most numerous after 6 and 10 years of non-fertilisation, but decreased when the period of non-fertilisation continued. The epidermis/root hair feeding genus *Filenchus* and the migratory endoparasite *Pratylenchus* in the soil were most numerous after 6 years of non-fertilisation, but were abundant also in the other fields. However, when the number of *Pratylenchus* in the roots was included as well no significant difference in the mean abundance could be observed among the four fields. In field B, that was unfertilised for the last 10 years, nematode species from both the early and late successional stages were found resulting in an intermediate position between the other fields. A characteristic species for field B was *Subanguina* (presumably *S. radicicola*) that was found in high numbers in the roots (up to 7000 nematodes per g fresh root weight) and soil of samples taken in September 1995 to April 1996. After 23 (field C) and 28 years of non-fertilisation (field K) the nematode community was characterised by the presence of small nematode species such as *Paratylenchus veruculatus*, *Aglenchus agricola*, and *Lelenchus leptosoma*. A high dominance of *P. veruculatus* and the presence of *Hemicycliophora triangulum* characterised field C, whereas a higher abundance of *A. agricola* and *L. leptosoma* and the presence of *Boleodorus volutus* characterised the samples of field K (Table 2.2a). The numbers of endoparasites and ectoparasites generally decreased with time of non-fertilisation, whereas the numbers of

Table 2.2a. Mean annual density and biomass (µg) per 100 cm^3 of soil, and mean number of genera per sample of plant-feeding nematodes in the rhizosphere soil of dominant plant species in each field studied.

Site:		Field O	Field O	Field B	Field C	Field C	Field K	Field K	Field K
Years unfertilised:		6	6	10	23	23	28	28	28
Plant species:		Lp	Hl	Hl	Hl	Fr	Hl	Fr	Ao
Mean number of nematodes per 100 cm^3 extracted from soil fraction									
Heterodera spec. A	1a	92 a	50 a	0 b	0 b	0 b	0 b	0 b	+ b
Heterodera spec. B	1a	0	0	0	6	6	0	0	9
Subanguina spec.	1a/1b	1 b	0 b	245 a	0 b	0 b	0 b	0 b	2 b
Pratylenchus fallax/crenatus	1b	239 ab	319 a	139 bc	170 bc	107 cd	165 bcd	95 d	101 d
Helicotylenchus pseudorobustus	1c	343 b	454 a	456 ab	29 c	24 c	97 c	45 c	38 c
Longidorus elongatus	1d	55 a	58 a	0 b	0 b	0 b	0 b	0 b	0 b
Tylenchorhynchus maximus	1d	30 a	25 a	0 b	0 b	0 b	0 b	0 b	0 b
Tylenchorhynchus dubius	1d	181 a	196 a	7 b	0 b	0 b	0 b	0 b	0 b
Geocenamus nanus	1d	321 a	159 b	47 c	2 d	0 d	0 d	0 d	1 d
Paratylenchus nanus	1d	1338 b	2411 a	171 c	0 d	0 d	0 d	+ d	1 d
Trichodoridae spp.	1d	0 c	1 c	11 a	12 a	5 b	0 c	0 c	0 c
Hemicycliophora triangulum	1d	0 b	0 b	0 b	68 a	85 a	0 b	0 b	+ b
Gracilacus straeleni	1d	0	0	0	5	13	0	0	0
Paratylenchus veruculatus	1d	0 e	0 e	1063 b	2918 a	2964 a	1169 bc	451 d	763 cd
Ecphyadophora tenuissima	1d	2	0	0	1	2	6	0	1
Tylenchus arcuatus	1e	10 ab	27 a	0 b	0 b	0 b	0 b	0 b	0 b
Coslenchus polonicus	1e	5	25	0	0	0	0	0	0
Coslenchus costatus	1e	24 b	64 a	38 b	1 b	1 b	27 b	21 b	4 b
Malenchus bryophilus	1e	90 a	109 a	+ b	4 b	4 b	2 b	0 b	1 b
Aglenchus agricola	1e	102 e	200 e	252 d	1029 b	478 c	1314 a	1548 a	1593 a
Filenchus spp.	1e	1297 a	1508 a	183 d	449 bc	301 cd	372 bcd	390 bcd	1001 b
Lelenchus leptosoma	1e	1 d	4 d	20 d	99 bc	160 a	135 ab	109 abc	85 c
Boleodorus volutus	1e	0 d	0 d	0 d	0 d	0 d	132 a	43 b	23 c
Aphelenchus avenae	2/1e	247 b	299 a	37 c	2 d	0 d	0 d	1 d	0 d
Aphelenchoides spp.	2/1b/1e	226 def	277 cde	162 f	443 bcd	209 f	379 ac	472 a	456 ab
Ditylenchus spp.	2/1b	15	2	15	15	2	3	2	2
Other fungivores	2	142 bc	155 c	230 b	596 a	506 a	140 bc	185 b	181 b

Table 2.2a continued

Site:		Field O	Field O	Field B	Field C	Field C	Field K	Field K	Field K
Years unfertilised:		6	6	10	23	23	28	28	28
Plant species:		Lp	Hl	Hl	Hl	Fr	Hl	Fr	Ao
Plant feeders	1	4131 bc	5609 a	2633 d	4793 ab	4150 bc	3417 cd	2703 d	3621 c
sedentary endoparasites	1a	92 b	50 b	245 a	6 c	6 c	0 c	0 c	11 c
migratory endoparasites	1b	239 ab	319 a	139 bc	170 bc	107 cd	165 bcd	95 d	101 d
semi-endoparasites	1c	343 b	454 a	456 ab	29 c	24 c	97 c	45 c	38 c
ectoparasites	1d	1926 b	2850 ab	1299 c	3006 a	3069 ab	1175 d	451 e	765 de
epidermis/root hair feeders	1e	1530 bc	1936 abc	494 e	1581 c	945 d	1981 abc	2112 bc	2705 a
Fungal feeders	2/1	629 b	733 ab	445 c	1056 a	718 b	522 bc	661 b	639 b
other nematode groups		5530 a	5676 a	5861 a	3236 b	2712 c	3875 b	3845 b	3219 b
Total number of nematodes		10148 b	11863 a	8708 c	8489 cd	7073 de	7674 cde	7023 e	7298 cde
Mean biomass (µg) of nematodes per 100 cm^3 extracted from soil fraction									
Plant feeders	1	244 a	284 a	96 bc	96 b	81 bc	83 bc	62 c	79 bc
sedentary endoparasites	1a	3 b	2 b	15 a	+ c	+ c	0 c	0 c	+ c
migratory endoparasites	1b	4	6	2	3	2	4	2	2
semi-endoparasites	1c	27 a	34 a	36 a	2 b	1 b	7 b	3 b	2 b
ectoparasites	1d	173 a	195 a	29 c	54 b	57 b	20 cd	8 d	11 cd
epidermis/root hair feeders	1e	37 bd	47 bcd	14 f	37 de	21 e	52 ab	49 ac	64 a
Fungal feeders	2/1	10 bc	10 bc	15 b	39 a	27 a	7 bc	6 c	9 bc
Mean number of genera per sample									
Plant feeders	1	8.8 a	8.8 a	6.5 bc	6.9 b	6.4 bc	6.1 c	6.2 c	5.9 c
sedentary endoparasites	1a	0.6 a	0.5 a	0.3 ab	0.1 b	0.1 b	0 b	0 b	0.1 b
migratory endoparasites	1b	0.8 b	0.9 b	0.9 b	0.9 bc	0.9 b	0.8 bc	0.8 bc	0.7 c
semi-endoparasites	1c	0.8 ab	1.0 a	0.8 b	0.3 d	0.3 d	0.5 cd	0.5 c	0.5 c
Ectoparasites	1d	3.6 a	3.5 a	1.7 bc	1.9 b	1.6 c	1.0 d	0.8 d	1.0 d
epidermis/root hair feeders	1e	3.0 d	3.0 d	2.8 d	3.7 bc	3.6 bc	3.9 abc	4.1 a	3.7 b
Fungal feeders	2/1	3.0 ab	2.9 ab	3.3 a	3.1 ab	2.8 b	2.1 c	2.1 c	2.1 c

Abbreviations: Lp) *Lolium perenne*, Hl) *Holcus lanatus*, Fr) *Festuca rubra*, Ao) *Anthoxanthum odoratum*, 1a) sedentary endoparasites, 1b) migratory endoparasites, 1c) semi-endoparasites, 1d) ectoparasites, 1e) epidermis/root hair feeders, 1) plant feeders, 2) fungal feeders. +) present, but with abundance of <0.5 per 100 cm. Mean values with the same letter were not significantly different (P<0.05)

Table 2.2b. Mean annual density and biomass (µg) per 100 cm^3 of soil of plant-feeding nematodes extracted from roots of dominant plant species in each field studied.

Site:		Field O	Field O	Field B	Field C	Field C	Field K	Field K	Field K
Years unfertilised:		6	6	10	23	23	28	28	28
Plant species:		Lp	Hl	Hl	Hl	Fr	Hl	Fr	Ao
Mean number of nematodes per 100 cm^3 extracted from root fraction									
Heterodera spec. A	1a	16 a	8 a	0 b	0 b	0 b	0 b	0 b	0 b
Heterodera spec. B	1a	0	0	0	2	+	0	+	+
Subanguina spec.	1a/1b	+ a	1 a	1887 b	6 a	+ a	2 a	+ a	+ a
Pratylenchus fallax/crenatus	1b	338 a	449 ab	420 ab	650 b	534 b	709 ab	434 a	543 ab
Helicotylenchus pseudorobustus	1c	10 bc	20 ab	6 c	5 c	6 c	27 a	20 ab	19 ab
Longidorus elongatus	1d	2 a	1 a	0 b	0 b	0 b	0 b	0 b	0 b
Tylenchorhynchus maximus	1d	+ ab	1 a	0 b	0 b	0 b	0 b	0 b	0 b
Tylenchorhynchus dubius	1d	5 a	6 a	+ b	0 b	0 b	0 b	0 b	0 b
Geocenamus nanus	1d	2 a	2 ab	1 bc	0 c	0 c	0 c	0 c	0 c
Paratylenchus nanus	1d	4 b	8 a	1 c	0 c	0 c	0 c	0 c	0 c
Hemicycliophora triangulum	1d	0 a	0 a	0 a	1 b	+ a	0 a	0 a	0 a
Paratylenchus veruculatus	1d	0 d	0 d	5 cd	31 a	30 ab	34 abc	14 bc	20 b
Ecphyadophora tenuissima	1d	0	0	0	1	+	0	+	+
Tylenchidae	1e	514 a	366 b	100 e	230 c	144 de	178 ce	163 cd	311 cd
Aphelenchus avenae	2/1e	15 b	23 a	5 c	+ c	+ c	0 c	1 c	+ c
Aphelenchoides spp.	2/1b/1e	288 a	240 ab	166 c	162 cd	97 e	201 acd	204 bc	214 bd
Ditylenchus spp.	2/1b	28	12	10	23	4	6	4	6
Plant feeders	1	893 ab	861 ab	2419 a	927 ab	715 bc	951 bc	632 c	893 bc
sedentary endoparasites	1a	16 b	9 b	1887 a	8 c	0 c	2 c	0 c	0 c
migratory endoparasites	1b	338 a	449 ab	420 ab	650 b	534 b	709 ab	434 a	543 ab
semi-endoparasites	1c	10 bc	20 ab	6 c	5 c	6 c	27 a	20 ab	19 ab
ectoparasites	1d	15 ab	17 a	6 cd	32 ad	30 a	35 ab	15 bc	21 d
epidermis/root hair feeders	1e	514 a	366 b	100 e	230 c	144 de	178 ce	163 cd	311 cd
Fungal feeders	2/1	331 a	275 ab	181 c	186 c	100 d	207 bc	208 bc	220 b
other nematode groups		541 a	465 ab	382 b	219 c	115 d	194 c	117 d	152 cd
Total number of nematodes		1764 a	1601 ab	2982 a	1331 bc	930 cd	1351 bcd	957 d	1265 cd

Table 2.2b continued

Site:		Field O	Field O	Field B	Field C	Field C	Field K	Field K	Field K
Years unfertilised:		6	6	10	23	23	28	28	28
Plant species:		Lp	Hl	Hl	Hl	Fr	Hl	Fr	Ao
Mean biomass (µg) of nematodes per 100 cm^3 extracted from root fraction									
Plant feeders	1	25 b	23 b	123 a	20 b	15 b	21 b	14 b	20 b
sedentary endoparasites	1a	1 b	+ c	112 a	+ c	+ c	+ c	+ c	+ c
migratory endoparasites	1b	7	9	8	13	10	13	9	11
semi-endoparasites	1c	1 bc	2 ab	+ c	+ c	+ c	2 a	2 ab	1 ab
ectoparasites	1d	5 a	3 a	+ b	1 b	1 b	1 b	+ b	+ b
epidermis/root hair feeders	1e	2 a	9 b	2 e	5 cd	3 de	4 cde	4 de	7 bc
Fungal feeders	2/1	2 a	1 a	1 bc	1 b	+ c	1 bc	1 b	1 b

Abbreviations: Lp) *Lolium perenne*, Hl) *Holcus lanatus*, Fr) *Festuca rubra*, Ao) *Anthoxanthum odoratum*, 1a) sedentary endoparasites, 1b) migratory endoparasites, 1c) semi-endoparasites, 1d) ectoparasites, 1e) epidermis/root hair feeders, 1) plant feeders, 2) fungal feeders. +) present, but with abundance of <0.5 per 100 cm. Mean values with the same letter were not significantly different (P<0.05)

Table 2.3. Characteristics of adult females of the observed plant-feeding nematode taxa.

Nematode taxa	Biomass (µg)	Stylet length (µm)	Feeding mode[1]		Cell modification	Occurence[2]			
						O	B	C	K
Heterodera	45.00	29	sedentary	Endoparasite	syncytia	**		*	*
Subanguina spec.	5.34	15	sedentary	Endoparasite	hypertrophy		***		
Pratylenchus fallax/crenatus	0.07	15	sedentary	Endoparasite	no	***	***	***	***
Helicotylenchus pseudorobustus	0.26	26	sedentary	semi-endoparasite	no	***	***	*	**
Longidorus elongatus	9.79	87	sedentary	Ectoparasite	hypertrophy	**			
Tylenchorhynchus maximus	0.75	26	sedentary	Ectoparasite	no	*			
Tylenchorhynchus dubius	0.24	19	browser	Ectoparasite	no	**	*		
Geocenamus nanus	0.14	15	browser	Ectoparasite	no	***	*		
Paratylenchus nanus	0.04	29	sedentary	Ectoparasite	no	***	**		
Trichodoridae spp.	0.47	46	sedentary	Ectoparasite	no		*	*	
Hemicycliophora triangulum	0.42	78	sedentary	Ectoparasite	no			**	
Gracilacus straeleni	0.05	55	sedentary	Ectoparasite	no			*	
Paratylenchus veruculatus	0.03	15	sedentary	Ectoparasite	no		***	***	***
Ecphyadophora tenuissima	0.01	9	browser	Ectoparasite	no	*		*	*
Tylenchus arcuatus	0.34	17	browser	Ectoparasite	no	*			
Coslenchus polonicus	0.13	13	browser	Ectoparasite	no	*			
Coslenchus costatus	0.08	12	browser	Ectoparasite	no	*	*		*
Malenchus bryophilus	0.02	9	browser	Ectoparasite	no	**		*	
Aglenchus agricola	0.06	12	browser	Ectoparasite	no	**	***	***	***
Filenchus spp.	0.03-0.30	8-13	browser	Ectoparasite	no	***	**	***	***
Lelenchus leptosoma	0.03	9	browser	Ectoparasite	no		*	**	**
Boleodorus volutus	0.04	9	browser	Ectoparasite	no				**
Aphelenchus avenae	0.12	15	browser	Fungivore	no	***	*		
Aphelenchoides spp.	0.01-0.05	6-9	browser	Fungivore	no	***	***	***	***
Ditylenchus spp.	0.04-0.08	5-10	browser	Fungivore	no	*	*	*	*

1) after Yeates et al. (1993) and Wyss (1981) and references cited in these papers. Species are classified as sedentary feeders when they feed for long periods (several hours to days) and browsers when they feed for short periods (less than one hour) on the same feeding site.
2) Abundance classes: * >2; ** >50; and *** >200 nematodes per 100 cm^3 of soil

epidermis/root hair feeders increased. This successional pattern is most obvious in terms of the nematode biomass, since nematodes with a relatively large body size were more abundant in the nutrient-rich field O, 6 years after fertilisation stopped (Table 2.3). As a result of that the mean biomass of individual nematodes decreased with time of non-fertilisation (mean individual biomass of 0.051, 0.028, 0.020, and 0.024 µg in field O, B, C, and K respectively).

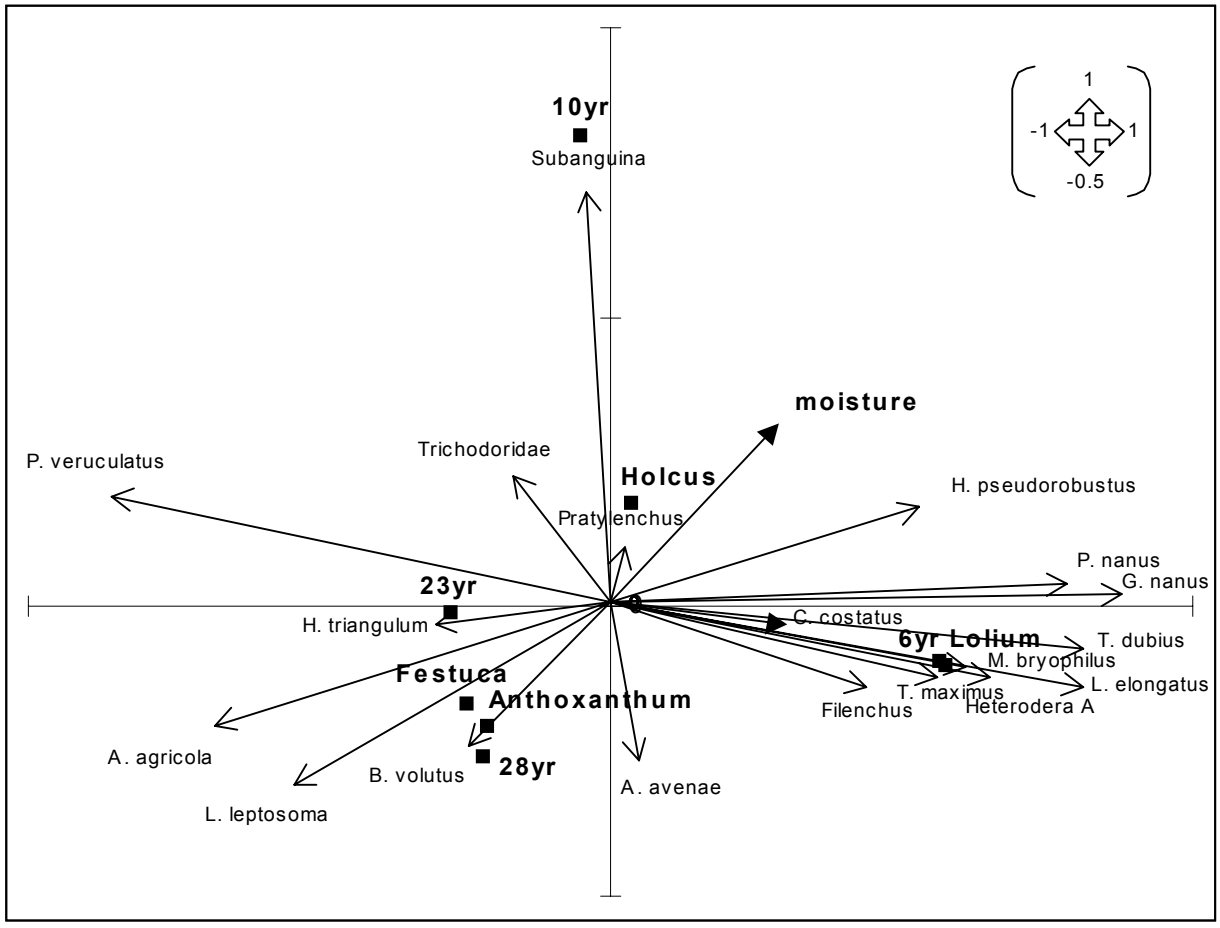


Figure 2.2. Correlation biplot of species and environmental variables (axes 1 and 2) based on a RDA of the nematode data from figure 1. The eigenvalues of the first four axes are 0.43, 0.05, 0.03, and 0.01, respectively. The sum of all canonical eigenvalues is 0.56. The nominal variables are presented by their centroids. Significant explanatory variables (Monte Carlo permutation test, $P \leq 0.05$) are presented only.

Direct ordination of the sites (RDA) (Fig. 2.2) resulted in a similar ordination pattern as the PCA (Fig. 2.1) and had eigenvalues of 0.432 for the first axis and 0.051 for the second axis. Monte Carlo permutation tests determined that both the first and second canonical axis, and all canonical axes together, explained a significant part of the variance in the dataset ($P \leq$

0.05). The similarity between both analyses is also shown by the high correlation between the PCA and RDA axes (r^2 = 1.000 for the first axis; r^2 = 0.983 for the second axis; r^2 = 0.939 for the third axis; r^2 = 0.756 for the fourth axis).

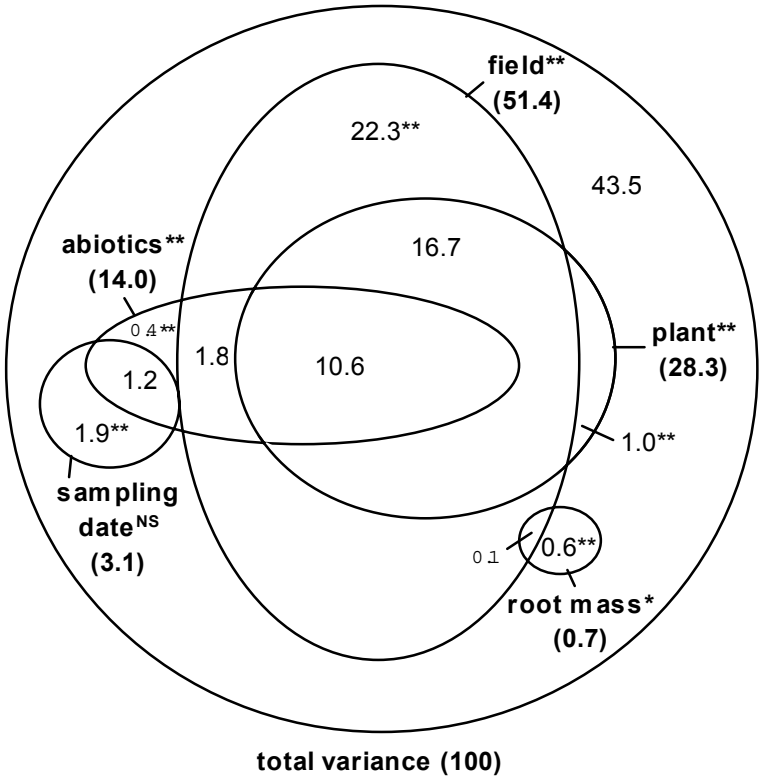


Figure 2.3. Schematic variance diagram based on the RDA of figure 2.2. For each of the explanatory variables it the percentage of the total variance of the nematode data explained is indicated. The outer circle represents the total variance (=100%) in the nematode data. The inner circles represent the variance explained by the different combinations of variables (bold letters). The total variance explained is 56.5%. NS = not significant, (i.e. P > 0.05); * = P ≤ 0.05; ** = P ≤ 0.01.

The contribution of each explanatory variable to the total amount of variance is visualised in Figure 2.3. All variables together explained 56.5% of the total variance in the species data. Most of the variance could be ascribed to differences in the nematode species composition between the field sites (51.4%). Part of the variance explained by field site could be ascribed to differences between fields in abiotic soil conditions (12.4%) and the composition of the plant community (27.3%). However, after removing the variance explained by differences between field sites only 1.6% of the variance could be ascribed to within field variation in

abiotic variables and 1.0% to differences between plant species. Besides spatial differences in biotic and abiotic environmental factors a small part of the variance could be ascribed to temporal differences in the nematode communities during the sampling season (3.1%), partly as a result of natural changes in climatic (temperature and precipitation) and soil conditions (moisture content). Root biomass explained only 0.7% of the variance.

RDA based on the total set of nematode data indicated a high amount of variance that was shared between the field sites and plant species. The variance could be attributed both to field site and plant species, as most plant species were sampled in one or two fields only. For example, all variance that is explained by the presence of *L. perenne* could be ascribed to the factor field as well, because this species was sampled in field O only. To circumvent this effect of the sampling set-up redundancy analyses were conducted for each field separately. For all four fields the eigenvalues of the axes and the total amount of explained variance (Fig. 2.4) was low, but Monte Carlo permutation tests determined that for each field both the first canonical axis (eigenvalue was 0.074, 0.126, 0.062, 0.074 after 6, 10, 23 and 28 years unfertilised, respectively) and all canonical axes together (sum of all eigenvalues was 0.212, 0.319, 0.208, 0.189 after 6, 10, 23 and 28 years unfertilised, respectively) were statistically significant at $P \leq 0.05$. In each field the set of explanatory variables explained about 20% of the variance. Most of the explained variance could be ascribed to sampling date (10.5-29.5%) which included the variance explained by the abiotic soil and climatic conditions (4.4-11.1%) completely. Plant species (2.4-6.2%) and root biomass (1.8-6.2%) had a low explanatory value, but for each field, in which two or more plant species were sampled, the contribution of the plant species was significant and the shared variance with other variables was negligible.

An RDA based on the nematode data that were collected under *H. lanatus* only, was performed to analyse the effect of fertiliser history on the nematode community within the rhizosphere of a single plant species. Such analysis resulted in an ordination pattern that resembled the ordination pattern for the total set of nematode data (see Fig. 2.2 and is therefore not shown separately). The eigenvalues were 0.397 for the first axis, 0.077 for the second axis, and 0.550 for the sum of all canonical eigenvalues. Monte Carlo permutation tests determined that both the first and second canonical axis and all canonical axes together were statistically significant ($P \leq 0.05$). Thus, the total set of explanatory variables explained55.0% of the total variance in the nematode dataset, of which 48.3% (*i.e.* 87% of all explained variance) could be ascribed to the time of non-fertilisation. The similarity between

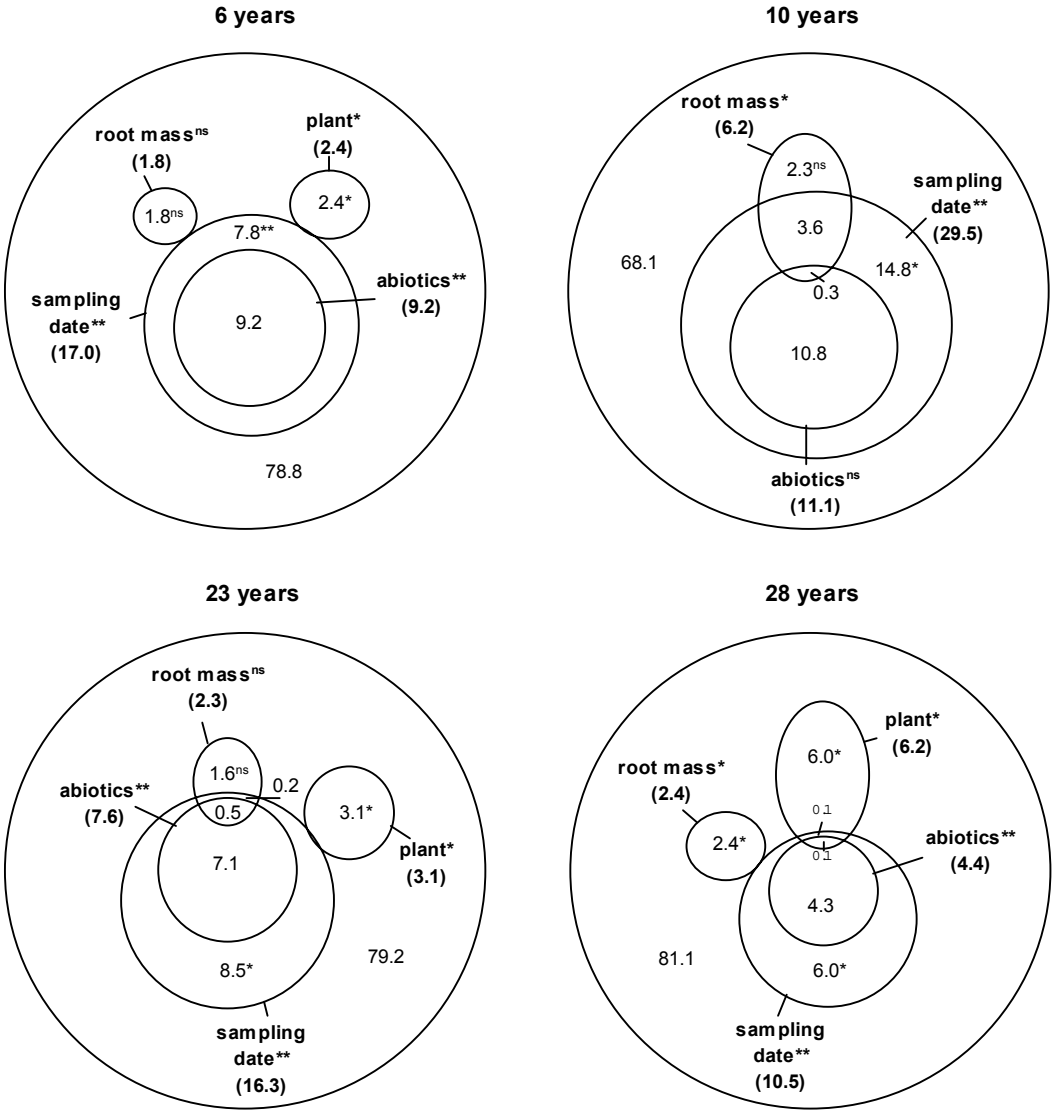


Figure 2.4. Variance diagram based on the RDA of the nematode data from the fields not fertilised for 6, 10, 23, and 28 years. See figure 2.3 for details.

the RDA results of the *H. lanatus* dataset and the total dataset is also shown by the high correlation between the axes of both analyses (r^2 = 1.00 for the first axis; r^2 = 0.97 for the second axis).

The species composition of nematode communities in the rhizosphere of coexisting plant species within each field showed a high resemblance (Table 2.2a/b), but variance analyses (MANOVA) revealed some significant differences in the composition of the nematode community between the rhizospheres of coexisting plant species (field O: $F_{11,73}$ = 2.03, P =

0.037; field C: $F_{10,74}$ = 2.15, P = 0.031; field K: $F_{20,180}$ = 1.71, P = 0.035). In field O significantly more nematodes of *H. pseudorobustus*, *P. nanus*, and *A. avenae* were found in the rhizosphere of *H. lanatus* than in the rhizosphere of *L. perenne*, whereas the opposite was found for *G. nanus*. In total 36% (18% in terms of biomass) more plant-feeding nematodes were found in the rhizosphere of *H. lanatus* compared to *L. perenne*. Also in field C the highest abundance of plant-feeding nematodes was associated with *H. lanatus*, but not statistically significant so compared to the nematode community associated with *F. rubra*. In field C *A. agricola, Aphelenchoides* spp., and the epidermis/root-hair feeders were significantly more abundant in the rhizosphere of *H. lanatus* than in the rhizosphere of *F. rubra*, whereas the opposite was found for *L. leptosoma*. In field K the highest mean abundance of plant-feeding nematodes was associated with *A. odoratum*, mainly because of a high number of epidermis/root-hair feeders. However, *B. volutus* and *P. veruculatus* were more abundant in the rhizosphere of *H. lanatus*.

Discussion

NEMATODE RESPONSE TO ENVIRONMENTAL CHANGES

Changes in the nematode community structure are constrained by the (apparent) lack of colonisation opportunities by which new taxa could enter the system in the available time. Nematode dispersal is, however, still poorly understood. Transport by wind is demonstrated by several authors, but generally in open systems such as arable land or sand dune areas (Krnjaic and Krnjaic, 1972; Yeates et al., 1991; Beaujard and Martiny, 1994). Particularly, in a closed grassland system the opportunities for wind-borne dispersal could be low. However, considering the fast colonisation of various nematode species in an abandoned field within five years after the eutrophic top-soil was removed (De Goede and Van Dijk, 1998), we assume that 28 years of non-fertilisation is sufficient time for nematode species to establish new populations also in grassland ecosystems.

The decrease in density and biomass of plant-feeding nematodes with increasing time of non-fertilisation coincided with the decrease in aboveground productivity (Olff and Bakker, 1991) and nutrient availability (Olff et al., 1994). This supports the observed positive relationship between nematode density and plant productivity (Yeates, 1979; 1987).

However, root biomass explained only a negligible part of the variance between the four fields (Fig. 2.3). Standing root biomass is not *per se* a good estimate of plant productivity as the turnover rate of root tissues is generally higher at nutrient-rich sites (Ryser, 1996a). Plant species of nutrient-rich sites generally have a higher nutrient uptake rate, a higher growth rate, a shorter tissue life expectancy and a lower tissue density than species of nutrient-poor sites (Ryser, 1996a,b). Since the palatability of plants is negatively correlated with the life expectancy of the plant organs (Southwood et al., 1986), a decrease in the number of plant-feeding nematodes could be expected as the period of non-fertilisation increased. A low palatability of slow-growing plant species can be the result of a lower N-content (Mattson, 1980) or an increased chemical defence (Edwards-Jones and Brown, 1993). Furthermore, the higher tissue density of slow-growing species can be a mechanical defence mechanism that encumbers the feeding of nematodes. Therefore, qualitative changes in the food source, which resulted from a reduced nutrient availability in the soil, likely affected the density of plant-feeding nematodes in the studied grasslands more than the standing root biomass of the vegetation.

Reproduction rates of plant-feeding nematode species generally depend on the host plant species. Therefore, changes in the vegetation composition likely result in a shift in the composition of the plant-feeding nematode community. Species-specific interactions of plants with their associated soil communities have been demonstrated in a succession of foredune vegetation (Van der Putten et al., 1993). In the foredunes, some nematode species, such as *Heterodera arenaria*, showed considerable specificity (Van der Putten and Peters, 1997; Van der Stoel and Van der Putten, unpublished data). The results of the redundancy analyses gave some support to our hypothesis that plant-specific nematode communities were present in the rhizosphere of each plant species. This conclusion was supported by the statistical analysis at community and species level, which revealed small but significant differences in nematode abundance between the rhizospheres of coexisting plant species (Table 2.2a).

In contrast to the small differences in the composition of the nematode community between different plant species within each field, the nematode community in the rhizosphere of *H. lanatus* markedly changed as the period of non-fertilisation increased. This indicates that environmental conditions and intraspecific rather than interspecific changes in food quality may affect nematode succession in grassland communities. However, the method we used for the nematode sampling might have been inefficient for detecting plant-specific nematode communities. Our intention was to sample the rhizosphere of single grass species,

but as clumps were generally small and roots of neighbouring species could have intermingled it is likely that most soil samples contained nematode communities of neighbouring plant species also. Furthermore, the number of plant species that was sampled within each field was limited to the two or three dominant grass species and ignored all species-specific interactions with other plant species of the grassland community.

Intraspecific differences in food quality could be the result of altered concentrations of both nutrients and defensive compounds in the plant tissues. Olff et al. (1994) reported that the concentration of nutrients (especially P) in plant tissues decreased after fertilisation was stopped. Moreover, intraspecific changes in the nutrient concentration were found (Olff, 1992a). Both the N and P content in leaf tissue of *H. lanatus* decreased during succession, whereas the N:P ratio increased. As nitrogen is generally regarded as a limiting factor for many herbivores, including nematodes (Mattson, 1980; Seastedt, 1985), a higher C:N ratio will reduce the number of nematodes. Furthermore, a higher C:N ratio might indicate a higher level of defensive compounds in plant tissues (Bryant et al., 1983; Hartley et al., 1995) which might deter plant-feeding nematodes (Yeates, 1987; Merrill et al., 1994).

Besides differences in the food source of the plant-feeding nematodes the environmental conditions such as organic matter content, acidity, and soil texture in the soil differed among the examined fields. Particularly, the soil pH in field O ($pH(H_2O) = 4.9$) was significantly higher than in the other fields ($pH(H_2O) \approx 4.0$). Changes in the soil pH can have a direct effect on the nematode community by affecting the exchange of ions through the nematode cuticle, or an indirect effect by affecting the food availability, or the (a)biotic environment (Korthals et al., 1996). However, changes in soil pH are often correlated to other factors such as plant and litter quality, through which observed relationships between pH and nematode density could easily be misinterpreted. In our opinion, changes in food quality should be considered when explaining pH effects on nematode communities.

The sampling date explained a significant part of the variance in each field (10-30%). The nematode community could be directly affected by changes in the abiotic environment or indirectly by the effect of abiotic changes on the quantity and quality of the food source, and by interfering with the biotic interactions with other organisms. Generally, soil moisture and temperature are mentioned as major factors affecting seasonal population fluctuations (Norton and Niblack, 1991). At least 38.7% of the seasonal variance in our dataset could be ascribed to the abiotic factors temperature, moisture and precipitation.

ADAPTATION OF FEEDING STRATEGIES BY NEMATODE SPECIES

Especially for nematode species with high nutrient and energy needs, a decrease in food quality can have considerable effects on the population size. Particularly, relatively large-sized nematode genera such as *Longidorus*, *Heterodera*, *Helicotylenchus* and *Tylenchorhynchus* declined or even disappeared when the nutrient availability decreased, whereas smaller nematode taxa such as *Aglenchus*, *Filenchus*, *Paratylenchus* and *Pratylenchus* were abundant in all sites and dominated in the nutrient-poor sites (table 2.3). As a result of the disappearance of the relatively large nematode species with time of non-fertilisation the nematode diversity decreased from about nine plant-feeding genera per sample in field O to six in field K.

The positive relationship between body size and nutrient availability is in contrast to the idea that, because of a more efficient energy use, a large body size is an advantage at low plant N concentrations (Mattson, 1980; Peters, 1983). Atkinson (1985) demonstrated that the mode of feeding based on the removal of contents from individual cells is a major constraint on size for many tylenchids. He suggested that the number of cells that can be attacked each day by a browsing nematode is the critical factor that determines the nematode body size. Thus, in spite of a more efficient energy use, nematode body size is first of all constrained by the absolute amount of food necessary for growth and maintenance. It seems that a reduced quality of the cell content, in terms of nutrient concentrations, also constrains the nematode body size, because more cells have to be attacked each day to acquire the same amount of nutrients.

Plant-feeding nematodes have developed several adaptations of their mode of feeding that support a large body size, either by reducing the costs of respiration and maintenance or by reducing the amount of energy being used in the uptake of food (Ferris, 1982). Taxa that possess a long stylet such as Longidoridae, Hemicycliophoridae, and Criconematidae can feed on columns of cells at the same feeding site (Wyss, 1981; Trudgill et al., 1991), thus reducing their costs of respiration and maintenance by spending less energy on movement. Similarly, a (semi)-endoparasitic feeding behaviour enables nematodes such as *Helicotylenchus*, *Rotylenchus*, *Amplimerlinius, Criconemoides* and cyst nematodes to feed on deeper cell layers (Taylor, 1961; Klinkenberg, 1963; Bridge and Hague, 1974; Wyss, 1981; Orion and Bar-Eyal, 1995). Furthermore, nematodes with a long stylet or an endoparasitic feeding behaviour can feed on higher quality tissues such as phloem cells (Wyss, 1981; Böckenhoff et al., 1996). This sedentary mode of feeding might explain why relatively large

species such as *H. pseudorobustus* and *H. triangulum* could survive in the nutrient-poor grasslands of field C and K, whereas the ectoparasitic browsers *G. nanus* and *T. dubius*, which are even smaller than *H. pseudorobustus* and *H. triangulum,* were restricted to the nutrient-rich fields O and B (Table 2.3).

In spite of a supposed more efficient utilisation of nutrients by endoparasites, the proportion of endoparasites (in the lumped density of plant-feeders of the soil and root fraction) did not increase (20.4, 62.4, 14.6, 18.6% in fields O, B, C and K, respectively) when the nutrient availability decreased. Only the proportion of the migratory endoparasite *Pratylenchus*, which has a small body size, increased at lower nutrient availability (11.7, 11.1, 13.8 and 16.4% in fields O, B, C, and K respectively). The relatively large body size of the dominant (semi-)endoparasites in the nutrient-rich fields (*Heterodera*, *Subanguina* and *Helicotylenchus*), likely acted as a trade-off for their density at a lower nutrient availability in spite of the energetic advantage of their endoparasitic feeding behaviour. However, within populations of the migratory and semi-endoparasitic nematode species, the percentage of nematodes that were extracted from the roots with regard to their total populations in the samples increased with time of non-fertilisation (59, 73, 82, and 82% of the *Pratylenchus* population and 4, 1, 19, and 29% of the *Helicotylenchus* population in fields O, B, C, and K respectively), indicating that endoparasitism is indeed an advantage at a low nutrient availability.

Perhaps the most specialised adaptation to a reduced nutrient availability is the formation of modified feeding cells (syncytia) that warrant a continuous supply of nutrients to the nematode at a small energy investment (Ferris, 1982). Such adaptation enables the large endoparasitic cyst- and root knot nematodes to complete their whole life cycle on one feeding site. The presence of *Heterodera* in the late successional fields C and K demonstrates their ability to maintain populations under poor food conditions despite a large body size. Similarly, root gall-forming nematodes of the genus *Subanguina* induce hypertrophy of parenchymatous tissue that might support a sufficient nutrient supply for maintenance of their large body size. Feeding cell modification is also known for the largest ectoparasitic feeding nematode taxa *Longidorus* and *Xiphinema* (Wyss, 1981; Trudgill et al., 1991). They induce root-tip galls containing multinucleate, enlarged cells that enable feeding for several days on the same feeding site. Contrary to the endoparasitic *Heterodera* and *Subanguina*, the ectoparasitic *L. elongatus*, was restricted to the nutrient-rich field O only. Modified feeding cells seem indispensable for such large nematodes to complete their life cycles, but

apparently could not supply enough food for *L. elongatus* populations to survive at a lower nutrient availability.

Conclusions

In line of our hypothesis the number and biomass of plant-feeding nematodes decreased with decreasing productivity of the grasslands. Furthermore, nematode species of nutrient-poor habitats in general had a relatively small body size or were characterised by energetically efficient feeding strategies such as endoparasitism, the ability to modify feeding cells, and a longer stylet. Such characteristics enable nematodes to sustain a relatively large body size even under nutrient-poor conditions. We found only small differences in the composition of the plant-feeding nematode communities between different plant species within each grassland. The composition of the nematode fauna under *H. lanatus*, however, considerably changed with time of non-fertilisation. We suggest, therefore, that qualitative changes within plant species and environmental conditions, rather than qualitative differences among plant species, affected the succession of the plant-feeding nematode community with decreasing nutrient availability of the soil.

VERSCHOOR, B.C., DE GOEDE, R.G.M., DE HOOP, J.W., DE VRIES, F.W. (2001) Seasonal dynamics and vertical distribution of plant-feeding nematode communities in grasslands. *Pedobiologia* 45: 213-233.

3. Seasonal dynamics and vertical distribution of plant-feeding nematode communities in grasslands

Abstract The vertical distribution and seasonal dynamics of plant- and fungal-feeding nematode taxa in permanent grasslands were investigated. Dolichodoridae, *Paratylenchus*, *Pratylenchus*, Tylenchidae and *Aphelenchoides* dominated the upper 10-cm soil and their numbers strongly decreased with depth. The vertical distribution of these nematodes was correlated with the distribution of roots in the soil profile. *Longidorus elongatus*, *Helicotylenchus pseudorobustus* and *Aphelenchus avenae* were, however, also prevalent in the deeper soil layers. Trichodoridae and *Hemicycliophora thornei* seemed to prefer a depth of 30-40 cm.

Most of the plant-feeding nematode taxa had an annual cycle in their abundance and population structures, which could largely be related to seasonal changes in the temperature and moisture contents of the soil. Although adult stages of sedentary endoparasitic genera were not counted, the peak number of juvenile *Heterodera* and *Subanguina* nematodes in spring indicated a distinct annual population cycle for these genera. The lowest densities of the semi-endoparasite *Helicotylenchus pseudorobustus* and the ectoparasites *Geocenamus nanus*, *Tylenchorhynchus dubius, Paratylenchus nanus, Paratylenchus veruculatus* and, to a lesser extent, *Tylenchorhynchus maximus* were found in winter/early spring, but their densities increased in summer/autumn. Generally, the population growth of these species in summer was preceded by a large proportion of adults followed by an increased number of juveniles. Taxa with short life cycles that could produce more generations per year, such as Tylenchidae and Aphelenchidae, and taxa with generation times of more than a year, such as *Longidorus elongatus,* generally did not show a distinct annual cycle.

Introduction

Plant-feeding nematodes are notorious pests in agricultural crops and (semi)-natural ecosystems. Besides their detrimental effects on plant growth, plant-feeding nematodes can have a substantial impact on nutrient dynamics in the soil, either negatively by reducing plant nutrition and productivity (Brussaard et al. 1996) or positively by enhancing root exudation and nutrient mineralisation (Bardgett et al. 1999a,b). To assess the impact of nematodes on plant and nutrient dynamics in ecosystems, it is essential to know the spatio-temporal dynamics of the nematodes themselves.

Nematode abundance and population structure changes during the season. Annual cycles differ between species and between years depending on the life history of species, food availability and quality, biotic interactions with other organisms, and the physical-chemical environment (Norton and Niblack 1991). Soil moisture and temperature are frequently mentioned as major factors affecting population fluctuations (Norton and Niblack 1991). For plant-feeding nematodes, however, also host plant dynamics and productivity are important factors regulating their activity (Yeates 1982; Norton 1989).

Root biomass and activity, soil temperature and moisture content are known also as important factors affecting the vertical distribution of plant-feeding nematodes in the soil (Rössner 1972; Yeates 1982; Norton and Niblack 1991). Generally, the major part of the nematode community can be found in the densely rooted 0-20 cm soil layer. However, species that are sensitive for dehydration, such as Trichodoridae and Longidoridae are often found to prefer deeper soil layers (Wyss 1970; Rössner 1972; Boag et al. 1987; Weischer and Almeida 1995; Ploeg 1998). Vertical migration of nematodes during the season is also observed, and is probably largely controlled by physical-chemical soil factors such as moisture content, temperature and texture (Yeates 1982; Norton and Niblack 1991).

The aim of the present study is to describe seasonal population dynamics and vertical distribution of plant- and fungal-feeding nematodes in a series of grasslands ranging from low to high levels of productivity. The focus here will be on the population dynamics of single nematode species within each grassland site.

The relationships between seasonal changes in the occurrence of nematode species and environmental conditions were analysed using calculations of Pearson's correlation coefficient to compare the nematode density and the environmental factors (root biomass, soil

moisture content, temperature and precipitation). The vertical distribution of plant-feeding nematodes and its possible relationship with the root length distribution was analysed. Furthermore, the description of the vertical distribution enabled us to determine whether sampling from the upper 0-10 cm of the soil is sufficient for an accurate quantification of the nematode community in the soil.

We hypothesised that the seasonal variation in nematode populations is mainly correlated to changes in temperature and soil moisture content, whereas the vertical distribution is mainly correlated to the distribution of roots.

Materials and Methods

SITE DESCRIPTION

The nematode community was surveyed in four hay-fields (O, B, C and K) located in the Drentse A Nature Reserve in the northern part of the Netherlands (53°N, 6°42'E) along a small stream, the Anloërdiepje. The soil in each field had a loamy sand texture. All grasslands were annually cut for haymaking in July/August. In each study site a topographic gradient was present ranging from the lower, wetter areas near the stream to the sandy plateau along the valley. The nematode survey was conducted on the highest and driest areas in each field. In the driest areas the vegetation in field O was dominated by *Lolium perenne*, *Holcus lanatus, Agrostis stolonifera*, and *Ranunculus repens*, in field B by *H. lanatus*, *A. stolonifera*, and *Anthriscus sylvestris*, in field C by *Festuca rubra, H. lanatus*, and *Agrostis capillaris*, and in field K by *F. rubra, A. capillaris, Anthoxanthum odoratum*, mosses and to a lesser extent *H. lanatus*.

SAMPLING

In each field, soil samples were collected from 10 x 15 m plots between September 1995 and November 1996. In total there were nine sampling times (01-09-95, 13-10-95, 30-11-95, 29-02-96, 17-04-96, 13-06-96, 7-08-96, 3-10-96, 27-11-96). At each sampling time 10 (field O), 5 (field B), 10 (field C), and 15 (field K) samples were randomly taken, using a split core sampler (diameter 47 mm) in the upper 10 cm of the mineral soil of each plot. In February 1996 the majority of the soil was frost-bound which obstructed the sampling. In February,

only 5 samples were, therefore, taken in fields O, C and K, and none in field B. A preliminary study showed that the large nematode species *Longidorus elongatus* occurred in field O only. In field O additional soil samples were, therefore, also collected for the extraction of *L. elongatus*. To study the vertical distribution of nematodes 5 soil samples were randomly taken in fields O and K to a depth of 40 cm in September 1995. Each of the samples was subdivided into four layers of 10 cm. All soil samples were transported intact and saved for a maximum of 10 days at 4°C before extraction.

Environmental conditions

In each field soil, the moisture content and soil temperature were measured at 10-15 cm depth with a datalogger in March-November 1996. Such data were missing for the first six months of sampling, therefore, the soil moisture content values were estimated from the differences between the soil fresh weight at sampling and the soil fresh weight of the samples taken in August 1996 when the soil was extremely dry. These estimates were found to be highly correlated with the actual moisture content (r^2 = 0.85, n = 20; based on data collected in March-December 1996). Daily air temperature and precipitation data were received from the local weather station in Eelde. The air temperature data were highly correlated to our measured soil temperatures (r^2 = 0.98, n = 60); based on data collected from March-December 1996). For statistical analysis the average daily air temperature and the precipitation over the last 21 days before sampling were used.

Extraction, counting and identification

All soil samples were weighed and soaked in a small amount of water prior to extraction. Nematodes were extracted from the soil suspension using a modified Oostenbrink elutriator (Oostenbrink 1960) and incubated for 48 hours (except the samples for vertical distribution, which were incubated for 30 hours) on a double cottonwool filter (Hygia milac filter). The remaining plant roots in the top sieve of the elutriator were collected, cleaned from soil particles, gently dried with tissue paper and weighed for fresh biomass. The roots were then cut into lengths of approximately 5 mm and macerated in a blender for 10 seconds. Endoparasitic nematode species were extracted from the macerated roots by means of the modified centrifuge flotation technique of Coolen and d'Herde (1972). *Longidorus* nematodes were extracted from soil using a modified Oostenbrink elutriator with a

continuous water up-stream of 1000 ml per minute. Instead of a cottonwool filter the *Longidorus* suspension was poured on a 175 μm sieve and incubated in an extraction dish for 48 hours.

After extraction 2.5-10% of the nematode suspension was counted under a low magnification inverted microscope. However, *Longidorus* samples were examined completely. Afterwards all the nematodes were heat-killed and fixed in 4% formaldehyde. The species composition of the plant-feeding nematode community of each soil sample was determined using a compound microscope (×400-1000). Nematodes collected from the root samples were identified in water suspensions using an inverted microscope (×100-400), because the presence of root fragments hampered slide preparation. Species nomenclature generally follows Bongers (1988), except for the genus *Merlinius*, which has recently been synonymised with *Geocenamus* (Brzeski 1991). The classification of feeding groups was made according to Yeates et al. (1993). Besides plant-feeders, the fungal-feeding nematodes were identified. These fungal feeders might affect the productivity of arbuscular mycorrhizas (AM) which we regard as an extension of the plant root system.

The roots collected from the 0-40 cm depth samples were not macerated for nematode extraction, but were weighed and used for the measurement of root length using the gridline intersect method of Giovannetti and Mosse (1980).

STATISTICAL ANALYSIS

Differences in the relative density of nematodes from the different soil layers were separated by the Least Significant Difference (LSD). Percentage data were arcsin transformed prior to analysis. The relationship between the vertical distribution of nematodes (numbers per soil volume) and root length was described using Pearson's correlation coefficient. To exclude differences in the horizontal distribution, due to considerable variance between the five replicates, the number of nematodes in each soil layer and the root length data were standardised by horizon (to percentage abundance).

The relationship between the seasonal changes in nematode density (per soil volume) and the environmental factors (temperature, precipitation, soil moisture content and root biomass) were analysed using Pearson's correlation coefficients. All nematode data were ln (x+1) transformed prior to analysis. Statistical analysis was performed only if the mean number of nematodes was >0.75 per gram of soil.

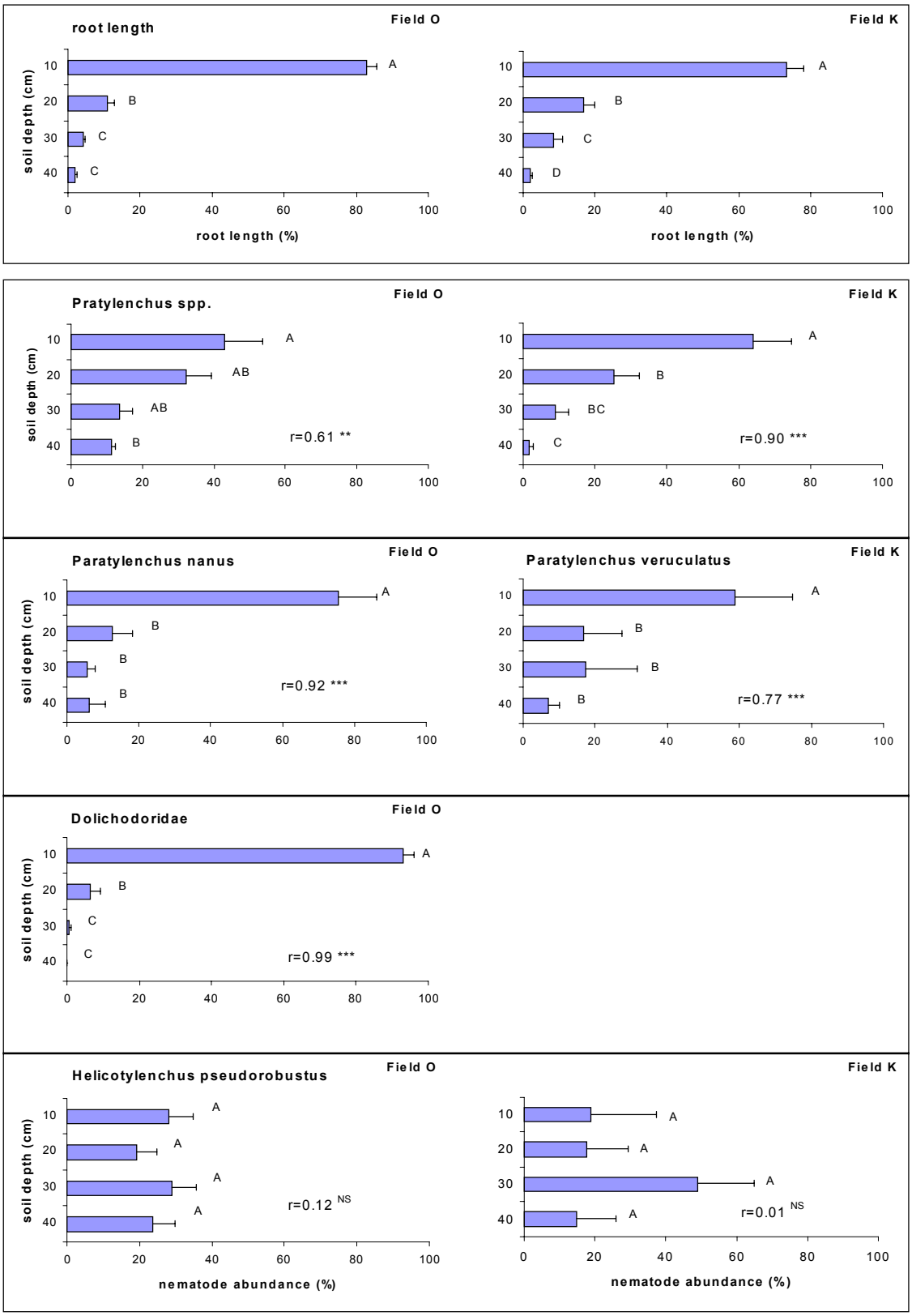
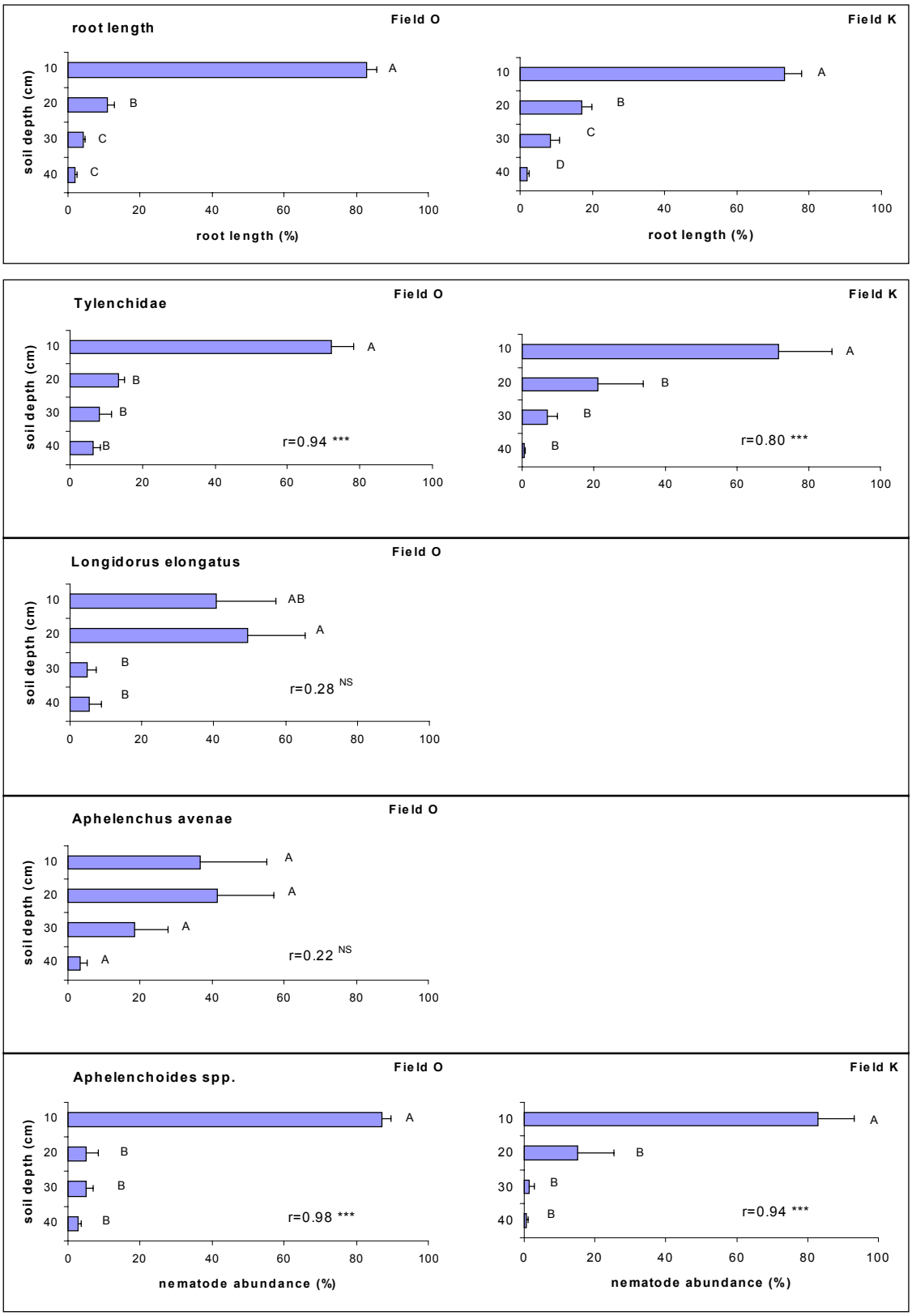


Figure 3.1. Mean (+ s.e.) density of nematodes and root length in each 10 cm soil layer expressed as percentage of the total density or root length in a 40 cm soil profile in two grassland sites (fields O and K).



Values with the same letters were not significantly different ($p > 0.05$). The correlation between the percentage nematode density and percentage root length is given by the coefficient r.

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Results

VERTICAL DISTRIBUTION

In total, 17 plant-feeding nematode taxa were identified in the samples taken for the vertical distribution study. For eight of the nematode taxa enough data were present to statistically analyse their vertical distribution. The relative abundance of the taxa in each soil layer and the relative distribution of root length are presented in Fig. 3.1.

More than 80% of the measured root length was present in the 0-10 cm soil layer and root length significantly decreased with depth. Likewise, nematode species of the genera *Paratylenchus*, *Pratylenchus*, *Aphelenchoides* and the families Dolichodoridae and Tylenchidae prevailed in the upper soil layer and decreased in numbers with soil depth. For all these nematode taxa a significant positive correlation was found with root length. The numbers of *Longidorus elongatus*, *Helicotylenchus pseudorobustus* and *Aphelenchus avenae* were not significantly different between the four soil layers and were not correlated with root length. However, *L. elongatus* and *A. avenae* tended to be more abundant in the 0-20 cm of soil, whereas *H. pseudorobustus* was evenly distributed over all soil layers. Two other plant-feeding nematode taxa, Trichodoridae and *Hemicycliophora thornei,* were also present in our samples, but their frequency was too low for statistical analysis. In total 13 specimens of Trichodoridae were found at a depth of 30-40 cm in contrast to 2 specimen in the 0-30 cm soil layer above. A large population (approx. 17,000 specimens) of *H. thornei* was found in a single sample from field K in the rhizosphere of a *Juncus effusus* plant. In this sample 2% of the *H. thornei* population was found in the upper 10 cm of soil, 3% at 10-20 cm depth, 28% at 20-30 cm depth and 67% at 30-40 cm depth.

SEASONAL CHANGES

Seasonal changes in precipitation, temperature, soil moisture content and root biomass in each field site are presented in Fig. 3.2. The soil moisture content was negatively correlated to the amount of precipitation (r = 0.34, $P < 0.05$) and positively to the mean daily temperature (r = -0.60, $P < 0.05$). No statistical relationship was found between the root biomass and these three environmental factors, but the root biomass was negatively correlated to the sampling time (r = -0.60, $P < 0.05$). Generally, the greatest root biomass was

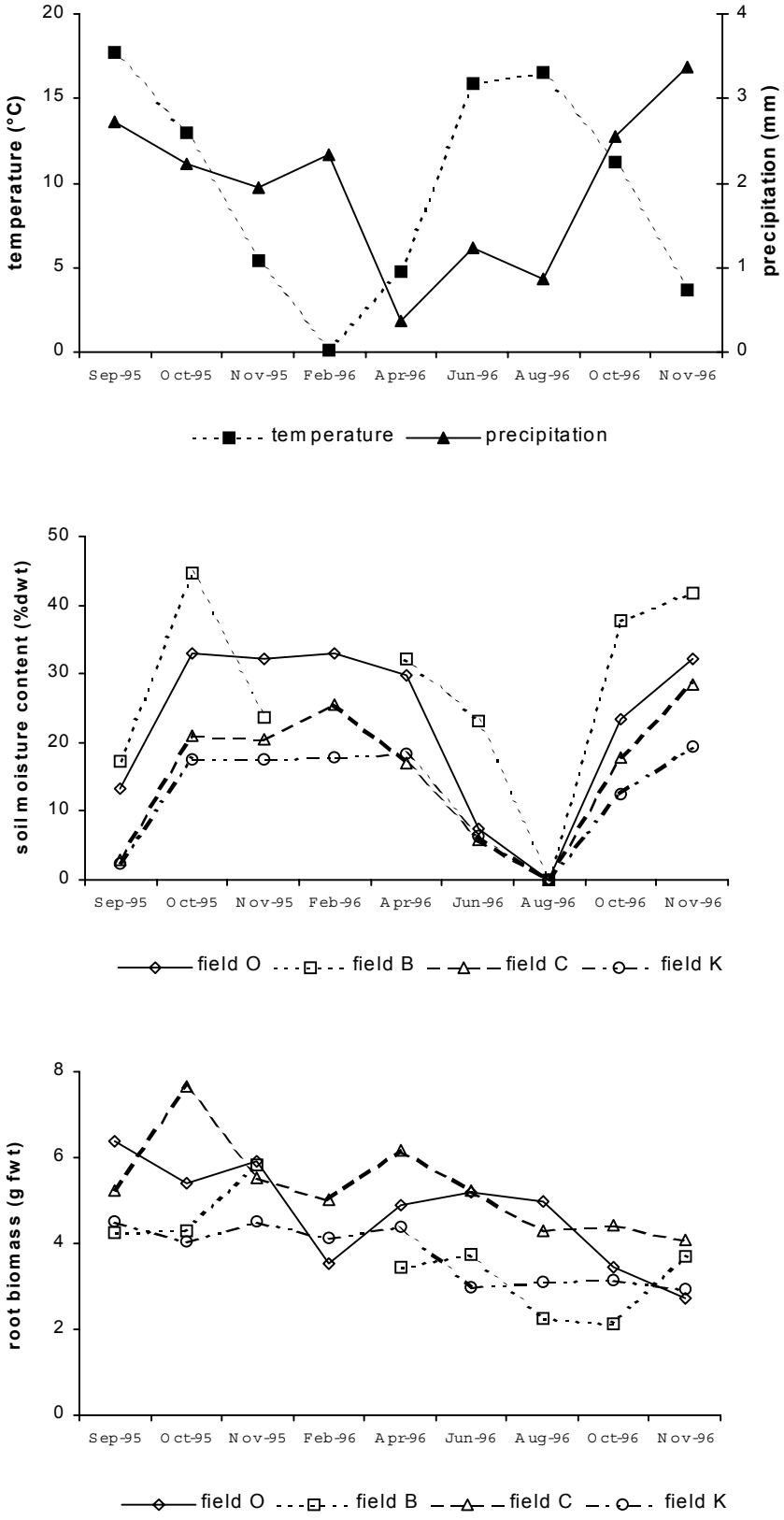


Figure 3.2. Seasonal dynamics in the mean precipitation (mm) and air temperature (°C) over a 21 day period before sampling, and soil moisture content (% soil moisture relative to August 1996) and root biomass (g fresh wt) in the upper 10 cm of soil from four grassland sites (field O, B, C, and K)

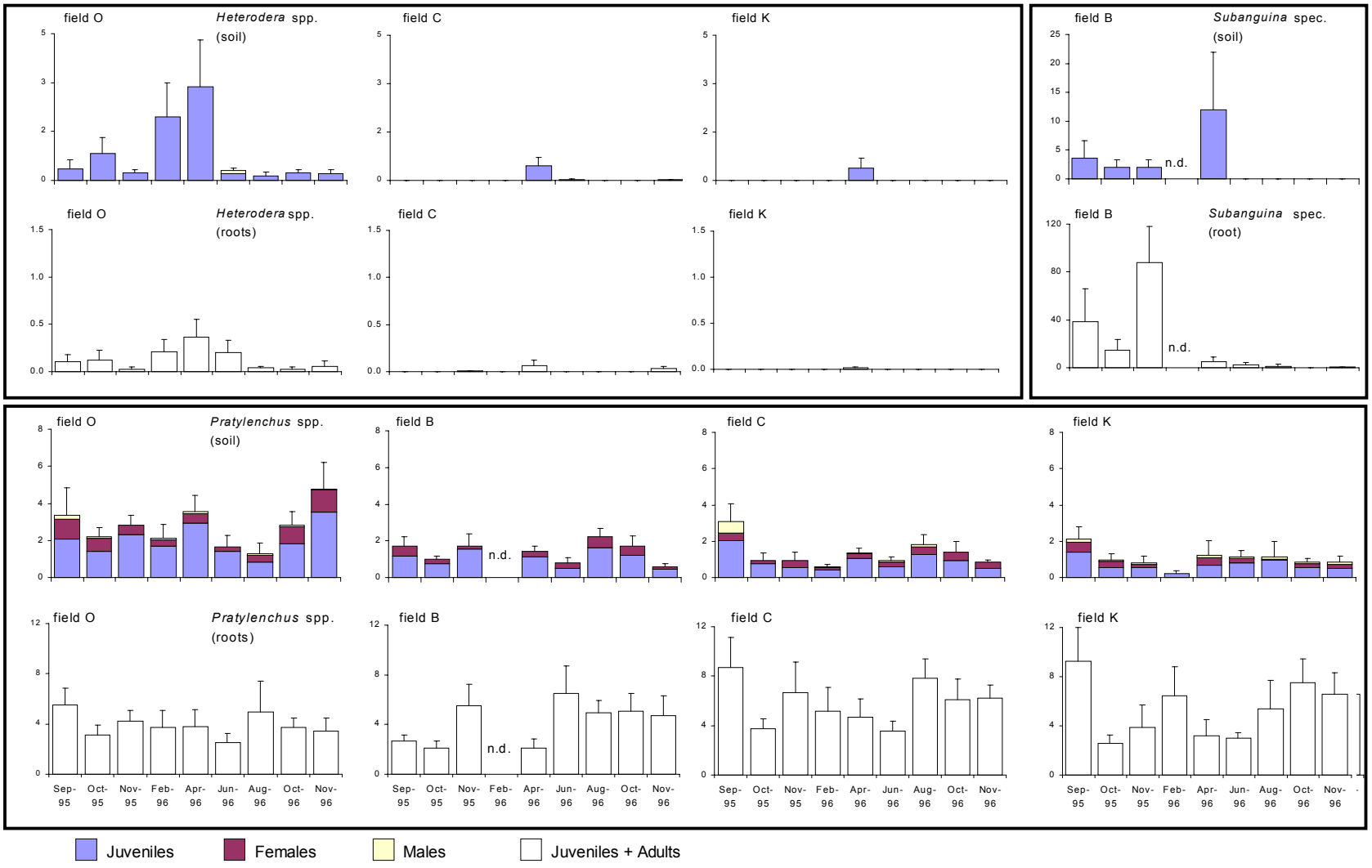


Figure 3.3. Seasonal changes in the mean (+ s.e.) number of nematodes in the soil and roots (both expressed per cm^3 of soil) for some plant- and fungal-feeding taxa from four grassland sites (fields O, B, C, and K). n.d. = not determined

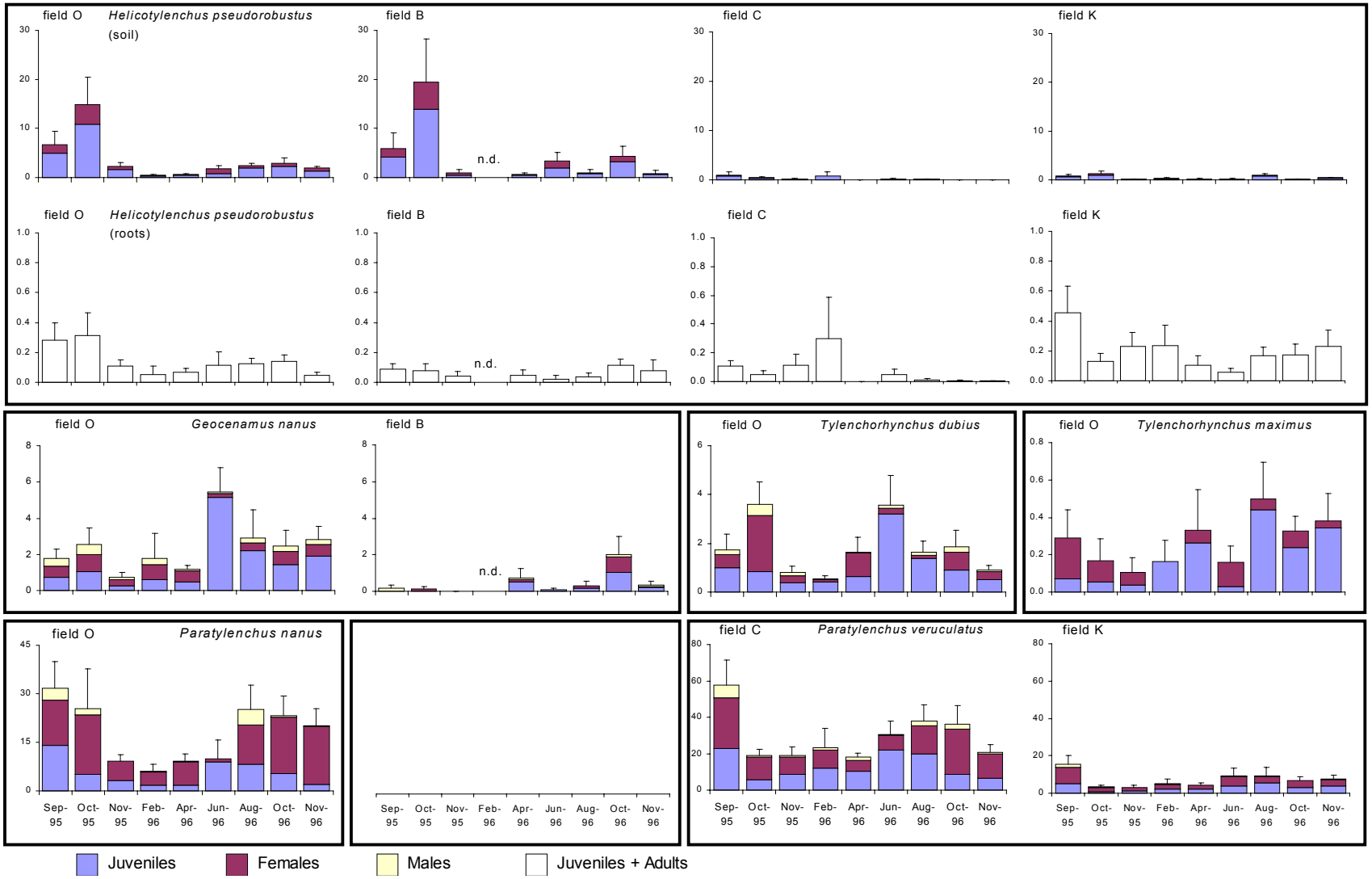


Figure 3.3 continued

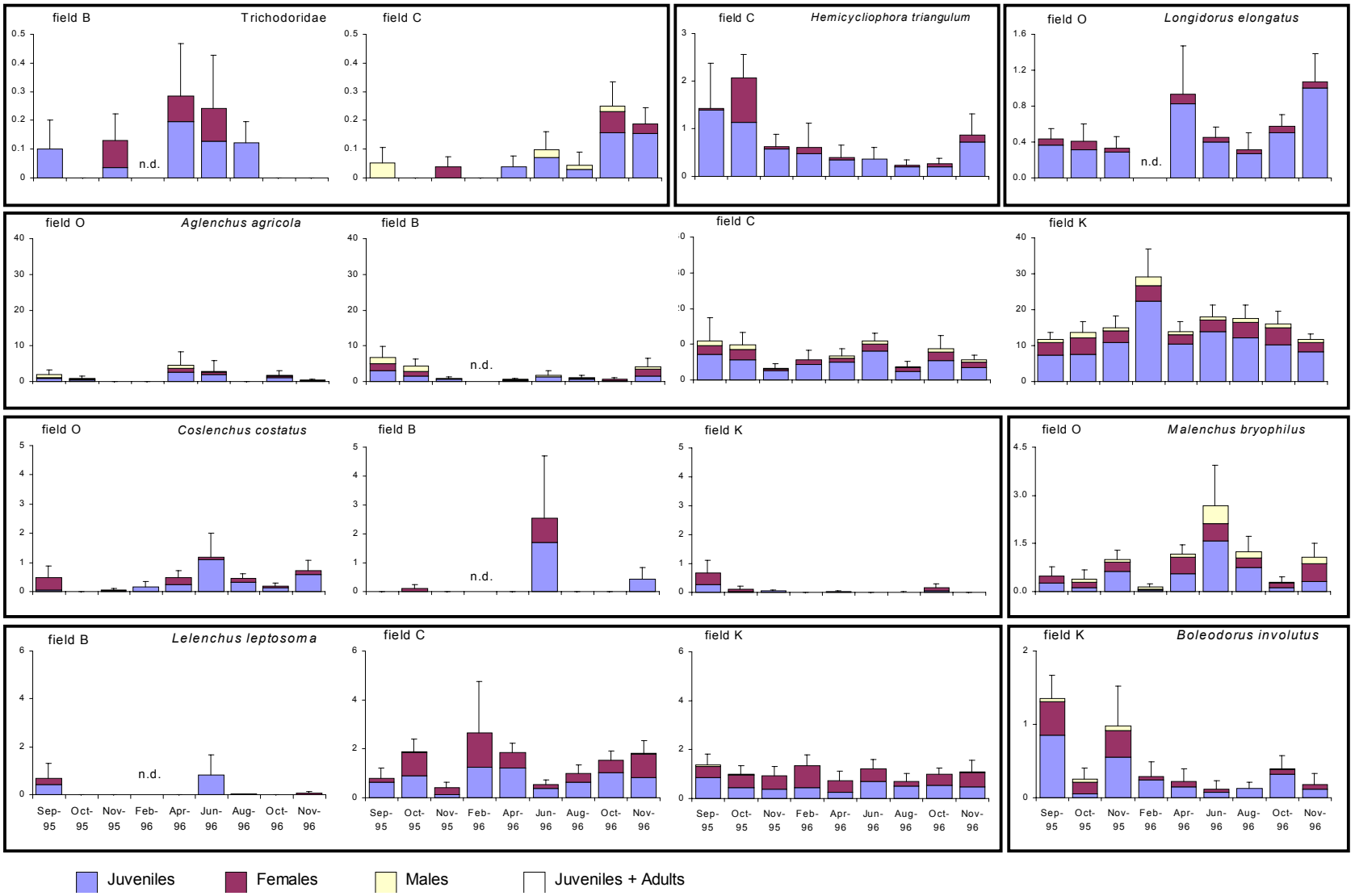


Figure 3.3 continued

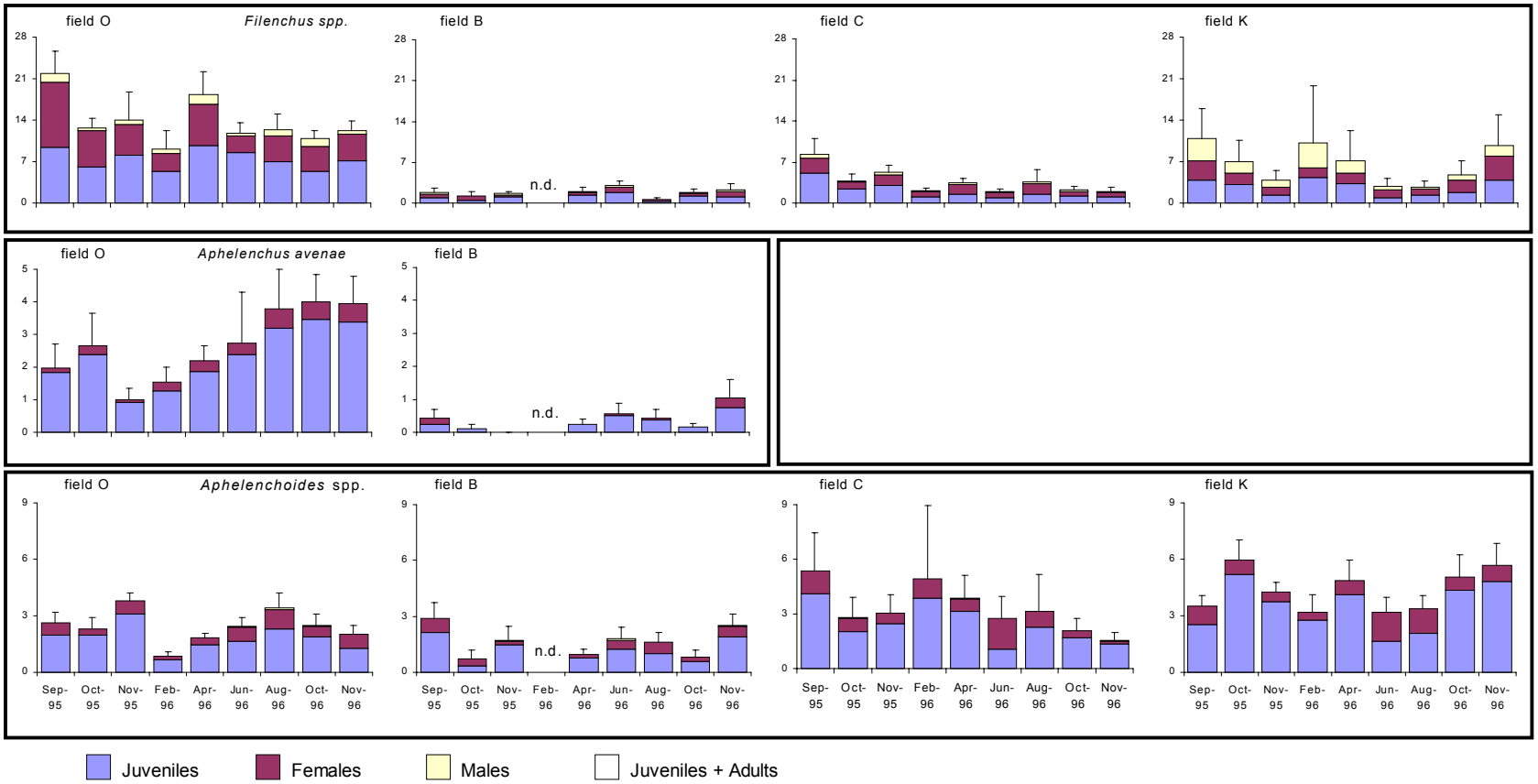


Figure 3.3 continued

found in the samples taken in 1995 and it then decreased during the course of 1996. This probably reflected the favourable weather conditions for plant growth in 1995 (relatively warm, an average annual precipitation and a gentle winter season), compared to those in 1996 (cold, harsh winter and a low average annual precipitation).

The seasonal changes in the mean numbers of nematodes of plant-feeding and fungal-feeding species and Pearson's correlation coefficients with the environmental factors are presented in Fig. 3.3 and in Table 3.1, respectively. In total 19 plant-feeding and 7 fungal-feeding genera were identified from the grasslands, but only the results of the dominant nematode genera or species are presented here.

The number of second-stage juveniles of the cyst nematode *Heterodera* peaked in early spring, from February to April 1996, and was low in the rest of the year. A few males were observed in the soil in June. No significant correlation was found between this species and any of the environmental factors.

Juveniles of the sedentary endoparasite *Subanguina* were found in large numbers in the roots (up to 7000 nematodes per g fresh root) in the autumn of 1995. In April 1996 most of these endoparasites had left the roots and were found in the soil. The density of *Subanguina* decreased to levels below the detection limit in the succeeding months of 1996. The density of *Subanguina* in the roots was positively correlated with root biomass.

The density of the migratory endoparasite *Pratylenchus* (mainly *P. crenatus* and *P. fallax*) in the soil decreased in winter. This was positively related to temperature (fields C and K) and negatively to soil moisture (field C). The number of *Pratylenchus* in the roots was positively correlated with precipitation in field K. The population structure of *Pratylenchus* in the soil was largely consistent during the year, though relatively more adults were found in the autumn in field O (35-38% adults in August-October 1995/1996 and 15-20% in November-June 1996). The percentage of the *Pratylenchus* population that was found in the soil peaked in September-October 1995 and April-June 1996 in fields B, C, and K.

The lowest densities of the semi-endoparasite *Helicotylenchus pseudorobustus* and the ectoparasites *Geocenamus nanus*, *Tylenchorhynchus dubius, Paratylenchus nanus, Paratylenchus veruculatus* and, to a lesser extent, *Tylenchorhynchus maximus* were found in the winter/early spring, but their densities increased in the summer/autumn. This is reflected by the positive correlation between the number of *H. pseudorobustus*, *T. dubius, P. nanus, P. veruculatus* and temperature. *P. veruculatus* was also negatively correlated to soil moisture content. *H. pseudorobustus* was a dominant nematode species in 1995, but its population decreased considerably in 1996, whereas the densities of *G. nanus* and both

Table 3.1. Pearson's correlation coefficient (×100) between the number of nematodes and the environmental factors (root biomass, soil moisture content, air temperature and precipitation). Correlation was calculated only when the nematode abundance was > 0.75 nematodes per gram soil. Significant correlations are given in bold italics and are marked with an asterix

Taxon	feeding type[1]	field	Root biomass	Moisture	Temperature	Precipitation
Plant-feeders						
Heterodera spec A	1a	O	-3	52	-50	-29
Subanguina spec (soil)	1a	B	60	13	-19	-19
Subanguina spec (root)	1a	B	***86*** *	-14	20	0
Pratylenchus spp.(soil)	1b	O	-25	62	-46	51
		B	-18	-61	30	-37
		C	-12	***-74*** *	***69*** *	-12
		K	-2	-53	***73*** *	-20
Pratylenchus spp. (root)	1b	O	33	-27	19	3
		B	-10	-30	4	8
		C	-58	-31	16	27
		K	-2	-17	-6	***67*** *
Helicotylenchus pseudorobustus (soil)	1c	O	44	-15	***67*** *	35
		B	4	31	59	34
Geocenamus nanus	1d	O	-28	-53	50	6
Tylenchorhynchus dubius	1d	O	40	-43	***76*** *	-30
Paratylenchus nanus	1d	O	14	-35	***68*** *	34
		B	63	19	-13	6
Paratylenchus veruculatus	1d	B	-30	-24	-31	-21
		C	-43	***-75*** *	***72*** *	14
		K	-40	***-79*** *	60	16
Hemicycliophora triangulum	1d	C	57	30	0	54
Malenchus bryophilus	1e	O	28	-46	31	-49
Aglenchus agricola	1e	O	18	-12	42	-11
		B	31	17	31	63
		C	33	-17	43	19
		K	-6	1	-31	-21
Lelenchus leptosoma	1e	C	9	53	-46	19
		K	11	2	-4	66
Filenchus spp.	1e	O	65	-15	33	-17
		B	36	48	-33	27
		C	33	-40	37	-3
		K	44	43	-43	57
Fungal feeders						
Aphelenchus avenae	2	O	-49	-37	37	8
Aphelenchoides spp.	2	O	54	-44	61	-16
		B	29	-44	12	32
		C	28	-35	7	-30
		K	-1	63	-25	31

[1] feeding type classification according to Yeates et al. (1993): 1a) sedentary endoparasites, 1b) migratory endoparasites, 1c) semi-endoparasites, 1d) ectoparasites, 1e) epidermis/root-hair feeders, 2) fungal feeders

Tylenchorhynchus species were highest in the summer of 1996. A high proportion of adults (April/June) followed by an increase in density mainly due to an increase in juveniles (June/August) preceded the population growth of each species in the summer.
The population increases of the smaller nematode species *P. nanus, P. veruculatus, G. nanus* and *T. dubius* started about two months earlier (June) than for the larger species *H. pseudorobustus* and *T. maximus* (August). During population growth the proportion of adults finally increased again. For the sexually reproducing *G. nanus, T. dubius, P. nanus* and *P. veruculatus* the male to female ratio also increased during the summer and autumn. In April, just before the start of the population growth, the male-to-female ratio was 0.17-0.27 for *G. nanus,* 0.03-0.43 for *T. dubius,* 0.02-0.09 for *P. nanus,* and 0.02-0.06 for *P. veruculatus*. This increased in summer/autumn to 0.68-0.72, 0.33-0.85, 0.26-0.39, and 0.12-0.25 respectively.

Hemicycliophora triangulum was most abundant in the autumn of 1995 and gradually decreased up to November 1996 when it slightly increased again. This species was positively correlated with root biomass.

The mean density of *Longidorus elongatus* peaked in April and November 1996, but its variability was high and no clear seasonal trend could be found. Neither was there a correlation with any of the environmental factors.

Trichodoridae were found only in low numbers and included *Trichodorus similis*, *Trichodorus viruliferus* and *Paratrichodorus pachydermus*. No clear seasonal trend for the Trichodoridae was found because of their low abundance, high variability and differences in species composition between sites and samples.

In contrast to the endo- and ectoparasitic nematode species no clear seasonal changes in the populations of the epidermis/root-hair feeding nematode species of the family Tylenchidae and the fungal-feeding *Aphelenchoides* were found. Neither did we find any statistically significant correlation with any of the environmental factors. The density of the fungal-feeding *Aphelenchus avenae* gradually increased towards the end of 1996.

Discussion

VERTICAL DISTRIBUTION

The root system of plants is considered to be the most important biotic factor affecting the plant-feeding nematode fauna in the soil (Norton 1989; Norton and Niblack 1991). Generally, nematode numbers are positively related to plant productivity (Yeates 1982). The distribution of plant-feeding nematodes in the soil profile may, therefore, reflect the distribution of the plant roots (Yeates 1987; Assheuer and Roessner 1993). This root-dependent distribution of nematodes was supported by the positive correlation between the vertical distribution of the relative root length and the relative numbers of several plant-feeding and fungal-feeding nematodes in our study. Contrary to our findings, Assheuer and Roessner (1993) found only weak correlations between the vertical distribution of root biomass and the number of plant-feeding nematodes. They concluded, therefore, that root biomass is of only minor importance as a determinant of the abundance of plant-feeding nematodes. Their study, however, differed in three ways to ours. Firstly, Assheuer and Roessner (1993) used absolute numbers of nematodes and root biomass instead of relative data. The standardisation of our data to percentages reduced the variance between the five replicates (*i.e.* horizontal variation) that otherwise would mask the true vertical variation. Secondly, we used root length as a parameter instead of root biomass because it was supposed that the length of the root system better reflects the number of feeding sites for nematodes than does root biomass. In fact other root parameters such as the distribution of fine roots, root tips or root hairs might be of even more value as most plant-feeding nematodes have a preference for certain root tissues. For example, the specific root length (i.e. root length/biomass), indicating the fineness of the roots, at 10-20 cm soil depth was almost twice as high as the specific root length at depths of 0-10 cm and 30-40 cm. Finally, our research sites were undisturbed permanent grasslands instead of arable fields in which the soil profile is frequently disturbed by tillage and other agricultural practices.

Nevertheless, the distribution of certain nematode species could not be explained by the distribution of the roots. *H. pseudorobustus* and to some extent *A. avenae* were distributed evenly over the 0-40 cm soil horizon. The trichodorid species and *H. thornei* even seemed to prefer a soil depth of 30-40 cm where very few roots were present. Experiments by Rössner (1970, 1972) showed that nematode species can have a preference for certain depths

independent of the presence of plants. Trichodorid and longidorid nematodes especially were found to migrate quickly to deeper soil layers and were also frequently found in deep soil layers (Boag 1981; Boag et al. 1987; Weischer and Almeida 1995; Ploeg 1998). Wyss (1970) demonstrated that tolerance to desiccation is probably an important factor affecting the vertical distribution of nematodes. Longidorid nematodes and especially trichodorids were very susceptible to desiccation (Wyss 1970), whereas *Tylenchorhynchus dubius*, which prefers the upper soil layers (this paper; De Maeseneer 1963; Wyss 1970; Sohlenius and Sandor 1987; Nombela et al. 1993), could withstand desiccation very well (Wyss 1970). Thus, migration towards deeper soil layers could be a strategy to escape from fluctuating moisture and temperature conditions in the upper soil layers despite a reduced availability of feeding sites. As for the Trichodoridae and Longidoridae, also *Hemicycliophora thornei* and *Helicotylenchus pseudorobustus* seemed to prefer deeper soil depths. Several species within the genera *Hemicycliophora* and *Helicotylenchus* have been found in deeper soil layers (up to 60 cm depth) in other studies (Luc 1961; Yuen 1966; Rössner 1972; Szczygiel and Hasior 1972; Yeates 1980; Sohlenius and Sandor 1987; Nombela et al. 1993). *Helicotylenchus,* however, generally seem to prefer a soil depth of 5-25 cm. It is likely, that their vertical distribution patterns are affected by the moisture and temperature conditions in the soil as well, but nothing is known about their tolerance for desiccation.

The results showed that by standard sampling of the upper 0-10 cm of soil in our hay-fields the majority of the plant-feeding nematode community was observed (60-70% of the total population at 0-40 cm soil depth). For some nematode taxa, however, such as *Helicotylenchus* (19-28%), *Longidorus* (41%), Trichodoridae (0%), and *Hemicycliophora* (0%), it is essential to sample at a lower depth for an adequate assessment of their population size.

SEASONAL CHANGES

Soil temperature and soil moisture content affect the activity of nematodes in the soil directly by altering the metabolic activity of nematodes (Atkinson 1980; Duncan and Klekowski 1975) or indirectly by altering the activity and quality of their food source (Yeates 1982). Soil temperature and moisture content are, therefore, considered to be the most important factors affecting the seasonal population dynamics of nematodes (Norton and Niblack 1991). Nevertheless, the number of significant correlations between nematode densities and environmental factors (Table 3.1) was low. This might suggest that soil temperature, soil

moisture, precipitation, and root biomass had a minor effect on the nematode numbers in the grasslands we examined. It is likely, however, that nematodes have a delayed response to environmental changes because it takes time for a population to develop. Furthermore, the activity and quality of the food source might be altered by temperature and moisture content as well. Such a species-specific lag-period in the response of nematodes should be taken into account in correlation analyses. To estimate the lag-period for each species it would be necessary, however, to sample more frequently than once every eight weeks. Moreover, it is likely that combinations of environmental factors affected the nematode densities in the field as opposed to single factors. Nevertheless, the results revealed some clear seasonal trends in the population dynamics of plant-feeding nematode taxa, which could reasonably be interpreted as changes affected by the environmental factors soil temperature and soil moisture content. Such correlations between nematode numbers and environmental factors have been demonstrated in long-term studies in New Zealand pastures, in which effects of year-to-year fluctuations could be examined more thoroughly than in the present study (Yeates and Risk 1976; Yeates 1982, 1984).

The peak density of second-stage juveniles of the cyst nematode *Heterodera* in spring indicates a life cycle of one generation per year as was described for the grass cyst nematodes *H. mani* and *H. bifenestra* in the Netherlands (Maas and Brinkman 1977, 1982). Sometimes a small second hatch of juveniles can occur in the autumn (Maas and Brinkman 1977, 1982). The emergence of juvenile *Heterodera* species is temperature dependent with an optimum of 13 °C (Maas and Brinkman 1977). Such conditions are typical for the spring when both temperature and root activity increase. After emergence from the cysts the juveniles enter the roots and grow into swollen sedentary females or free-living males. We could not follow the numerical changes of the *Heterodera* females because of the inadequacy of our extraction methods to extract such sedentary life stages, but some males were found shortly after the peak of second stage juveniles.

The endoparasitic nematode *Subanguina,* which was present in field B, forms root galls in which the second stage juveniles hibernate. After they leave the root galls in April, the juveniles attack new root tips of grasses and form new galls (Krall 1991). This is in agreement with our observation of a decrease in the numbers of juveniles found in the roots and an increase in numbers found in the soil during April 1996. According to Krall (1991) several generations of *Subanguina* have been observed in the Moscow region per year. In contrast, in our study site *Subanguina* declined dramatically after April 1996 to densities approximately the level of the detection limit.

The total numbers of the migratory endoparasite *Pratylenchus* (mainly *P. crenatus* and *P. fallax*) in the soil increased with temperature and decreased with soil moisture content in fields B, C, and K, whereas no such relationships were found for the numbers in the roots. The relatively high numbers of *Pratylenchus* nematodes in the soil compared to the root population, in September-October 1995 and April-June 1996 in fields B, C, and K, might indicate a high reproductive capacity during these months. In the winter season most *Pratylenchus* nematodes were found inside the roots in which they might be less affected by the adverse winter conditions. A lower abundance of *Pratylenchus* in the soil during the winter season was also found by Nombela et al. (1993) in arable fields. They frequently found *Pratylenchus* in the deeper soil layers during the winter and suggested that *Pratylenchus* migrated to deeper soil layers to escape from low temperatures. *Pratylenchus* can produce several generations per year (Loof 1991). The high numbers of *Pratylenchus* in the autumn of 1995 indicate the development of such a second generation in this year. The lack of an autumn peak in 1996 may have been due to a delay in the population development after an exceptionally cold and dry winter/spring.

The populations of the ectoparasitic nematodes *P. nanus*, *P. veruculatus*, *H. pseudorobustus*, *G. nanus*, *T. dubius* and *T. maximus* all appeared to have a distinct annual cycle related to the seasonal changes in temperature and moisture content of the soil. Similar annual cycles were found for related species of the family Dolichodoridae and Hoplolaimidae by Berge et al. (1973) in French grasslands. The sequential increases in the proportion of juveniles in the summer and the proportion of females and males in late summer/autumn indicate that these species reproduce at a rate of one generation per year. The mating of individuals of sexually reproducing species may take place in summer and autumn when the proportion of males is high. As the number of juveniles did not increase before June, the sperm must have been stored in the spermatheca of the females until spring. The late reproduction of *T. maximus* and *H. pseudorobustus* in August, as compared with June, for *P. nanus*, *P. veruculatus*, *G. nanus*, and *T. dubius* could be related to their larger body size. Generally, smaller nematode species have a shorter generation time as was shown for marine nematodes (Vranken et al. 1986). A longer generation time, reflected by a longer hatch period (17-19 days for *T. maximus* (Bridge 1974), 8 days for *H. pseudorobustus* (Taylor 1961) and *T. dubius* (Sharma 1971) and 5-6 days for a *Paratylenchus* spec. (Rhoades and Linford 1961)) and higher temperature requirements for reproduction (Malek 1980) probably delayed the reproduction of the larger nematode species during the season. More generations per year can be expected for *Paratylenchus* because of its shorter life cycle. For example, Berge et al.

(1973) noticed also a small second peak of a *Paratylenchus* species in the spring. In our study site no such spring peak was observed, that might have been due to the exceptionally cold and dry winter/spring of 1996.

The highest numbers of *Hemicycliophora triangulum* and *Longidorus elongatus* were found in October-November and in April and November, respectively, when the temperature was low and the moisture content of the upper soil layer was high. Possibly, these nematode species prefer the moist soil conditions in autumn and early spring for reproduction. On the other hand it might also indicate vertical migration from deeper soil layers which seems to be largely controlled by physical-chemical soil factors (Yeates 1982; Norton and Niblack 1991). Nematodes taxa that prefer deeper soil layers, such as trichodorid and longidorid nematode species were found to respond strongly to changes in soil moisture and temperature (Wyss 1970; Rössner 1970, 1972; Ploeg 1998). As mentioned before, also *Hemicycliophora* species are frequently found in the deeper soil layers (this paper; Luc 1961; Szczygiel and Hasior 1972) and may respond similarly to environmental changes as trichodorid and longidorid species.

No distinct seasonal cycle could be found for *L. elongatus*, which is probably the result of their long generation time of >1 year and low reproduction rate (Weischer and Almeida 1995). Species with short life cycles such as the root/fungal feeders of the family Tylenchidae and the fungal feeding genera *Aphelenchoides* and *Aphelenchus* did not show a regular annual cycle either. Their short life cycles and relatively low temperature requirements for reproduction (Hechler 1962; Goodey and Hooper 1965; Cayrol 1967; Gowen 1970; Moens et al. 1996) enable them to produce several generations per year that might obscure population dynamics in these taxa. Their capacity to reproduce at lower temperatures might also explain the relatively high abundance of Tylenchidae in the winter compared to the other plant-feeders. Furthermore, the proliferation of decomposer fungi on organic residues that have accumulated during winter (Bardgett et al. 1999c) might supply a useful food source in the early season for such root/fungal feeders.

VERSCHOOR, B.C. Carbon and nitrogen budgets of plant-feeding nematodes in grasslands of different productivity. Accepted by *Applied Soil Ecology*

4. Carbon and nitrogen budgets of plant-feeding nematodes in grasslands of different productivity

Abstract The amounts of carbon and nitrogen that are transferred from the plant biomass to the soil by the feeding activity of herbivorous nematodes in four grasslands were calculated. The grasslands differed in the length of time since last fertiliser addition, which resulted in a gradient of nutrient-rich, high-production to nutrient-poor, low-production grasslands. The biomass of plant-feeding nematodes decreased with time of non-fertilisation from, 200 mg dry weight m^{-2} after 6 years without fertilisation to 50 mg dry weight m^{-2} after 28 years without fertilisation. Consequently, the total CO_2 respiration rate of plant-feeding nematodes decreased with time of non-fertilisation from 4.5 to 1.7 l CO_2 m^{-2} $year^{-1}$. This corresponded to a carbon consumption of 15.95 to 6.13 g C m^{-2} $year^{-1}$ and a nitrogen consumption of 2.13 to 0.82 g N m^{-2} $year^{-1}$.

The direct contribution of plant-feeding nematodes to N mineralisation was approximately 2-5% of the total N mineralisation. It is suggested, however, that the indirect contribution of plant-feeding nematodes to N mineralisation may be much higher due to the release of relatively high amounts of organic nitrogen to the soil by defecation, biomass turnover and increased root exudation.

The biomass consumption of plant-feeding nematodes was approximately 3-5% of the total standing biomass in all four grasslands and 5-9% of the root standing biomass. Within a grassland, however, large local differences in the numbers of plant-feeding nematodes resulted in consumption values of 1-40% of the root standing biomass. These large differences in consumption values indicate that the spatial distribution of plant-feeding nematodes can be an important factor affecting vegetation dynamics.

Introduction

Nematodes are probably the most abundant group of multicellular soil animals in grasslands (Curry, 1994). Densities of 1-30 million nematodes m^{-2} are reported for grassland soils (Curry, 1994; De Goede and Bongers, 1998). Approximately 25-50% of all nematodes in grasslands are plant-feeders. These plant-feeding nematodes are considered to be a major factor affecting the net primary production of grasslands (Scott et al., 1979; Stanton, 1988). To illustrate this, several studies have shown that the application of nematicide resulted in considerable increases in the net primary production in American prairies of 25 to 59% (Smolik, 1977; Stanton et al., 1981; Ingham et al., 1986b; Ingham and Detling, 1990).

In contrast, plant-feeding nematodes may also play a beneficial role in the cycling of carbon and other nutrients from plant to soil. Plant-feeding nematodes contribute directly to nutrient mineralisation by the excretion of inorganic nitrogen such as ammonia. Efforts to estimate the carbon (C) and nitrogen (N) budgets of plant-feeding nematodes have, however, suggested a relatively low direct contribution of plant-feeding nematodes to the total N mineralisation in an arable soil (<1%) (De Ruiter et al., 1993). On the other hand, root herbivory by nematodes may have considerable indirect effects on nutrient mineralisation (Bardgett et al., 1999a,b). Nitrogen defecation by nematodes and nematode death increase the total N input to the soil. Furthermore, root herbivory can enhance root exudation of nitrogen to the soil. This organic N subsequently stimulates the microbial activity in the rhizosphere (Yeates et al., 1998; Yeates et al., 1999a; Denton et al., 1999). Consequently, both nutrient mineralisation and plant nutrient uptake can be increased as was shown by Bardgett et al. (1999a,b).

In the Drentse A nature reserve, The Netherlands, grasslands that were formerly managed according to standard agricultural practices typical for permanent grasslands, were set aside for restoration purposes. Initially, these grasslands had a high productivity due to the annual application of fertilisers. After cessation of fertilisation, however, their productivity has decreased and plants have to obtain their nutrients completely from the weathering of minerals, from the mineralisation of organic matter and from atmospheric deposition. Under such nutrient-limited conditions, soil organisms are particularly important as they contribute relatively more to soil nitrogen supply than under fertilised conditions (Brussaard et al., 1996).

In the present study, I calculated the C and N fluxes through plant-feeding nematodes in four grasslands that differed in the length of time since last fertiliser addition. On the basis of these values, I estimated the contribution of plant-feeding nematodes to C and N turnover and N mineralisation in these grasslands. Furthermore, I examined the consumption of plant biomass by nematodes in relation to the total standing plant biomass of each of the grasslands.

Calculation of carbon and nitrogen budgets

STUDY AREA

Nematodes were sampled from four grasslands in the Drentse A nature reserve, The Netherlands, that differed in the length of time since fertiliser was last added. The four grasslands (O, B, C and K) had not been fertilised for 6, 10, 23 and 28 years, respectively, and their productivity decreased in the same order. For detailed descriptions of these grasslands see chapter 2 (Verschoor et al., 2001a).

NEMATODE ABUNDANCE AND BIOMASS

The abundance, community structure and seasonal dynamics of plant-feeding nematodes in the four grassland sites were investigated by regular sampling in 1996 (chapters 2 and 3: Verschoor et al., 2001a,b). These data were used to calculate the contribution of the plant-feeding nematode community to C and N fluxes in the soil.

The numbers of nematodes of each plant-feeding species in the 0-10 cm soil layer are given in chapter 2 (Verschoor et al., 2001a). The numbers of nematodes in the deeper 10-40 cm layer were estimated on the basis of data given in chapter 3 (Verschoor et al., 2001b). All nematode counts were corrected for the size-specific extraction efficiency following Verschoor and De Goede (2000).

The fresh biomass of the nematodes was calculated using the formula of Andrássy (1956): $W\ (\mu g) = (B^2 \times L)/(16 \times 10^5)$. Adult body length was taken from measurements of Verschoor and De Goede (2000). Body width B was calculated from the ratio between L and the mean nematode L/B quotient (derived from Bongers, 1988). Biomass was calculated separately for

juveniles and adults. The mean juvenile length was assumed to be half the mean adult length, which is based on calculations of Verschoor and De Goede (2000). Fresh nematode biomass was converted to dry biomass assuming a dry-matter content of 25% (Yeates, 1979).

CARBON AND NITROGEN BUDGETS

The oxygen consumption rate of nematodes was calculated according to Klekowski et al. (1972). The oxygen consumption rate was calculated for juveniles and adults of each nematode species separately on the basis of their biomass using the formula $R=29.25*W^{0.72}$, where R is the amount of O_2 consumed (mm^3 per individual h^{-1}) and W is body weight (g fresh weight basis). Thereafter, the total oxygen consumption of the plant-feeding nematode community was calculated using correction factors for the soil temperature and soil moisture content.

Nematode oxygen consumption rates vary with environmental temperature T according to Krogh's normal curve (Duncan and Klekowski, 1975). A temperature correction was accomplished by multiplying the value R by $Q_{10}^{((T-t)/10)}$, where Q_{10} is assumed to be 3 (Didden et al., 1994), and t is 20°C. Unfortunately, the data on temperature fluctuations in the soil were incomplete. Therefore, I used the air temperatures, which were similar to the soil temperature at a depth of 5 cm (unpublished data). The mean, minimum and maximum daily air temperatures were received from the local weather station in Eelde. The fluctuations of soil temperature generally become smaller with increasing soil depth (Geiger et al., 1995). Furthermore, the mean soil temperature increases with soil depth during winter, whereas only slight differences in mean temperature with depth are found in summer (Geiger et al., 1995). Therefore, the temperatures in the deeper soil layers were derived from the surface temperature by assuming that in each 10-cm soil layer the temperature changed according to the formula $X_b = 0.7X_a+3.5$, in which X_b is the soil temperature in the 10 cm soil layer b below the 10 cm soil layer a. The adjusted daily temperature in each soil layer is presented in Fig 4.1.

The soil moisture content was measured fortnightly in the 0-10 cm soil layer of each field. Demeure et al. (1979) found that, in a loamy sand soil, nematodes entered anhydrobiosis at a water potential of –0.03 MPa and reached a maximum percentage of coiled nematodes at approximately -0.1 MPa. On the basis of these data the proportion of active nematodes was calculated as $1/[1 + (10^6 \times e^{-24m})]$, where soil moisture m is a fraction of the water holding

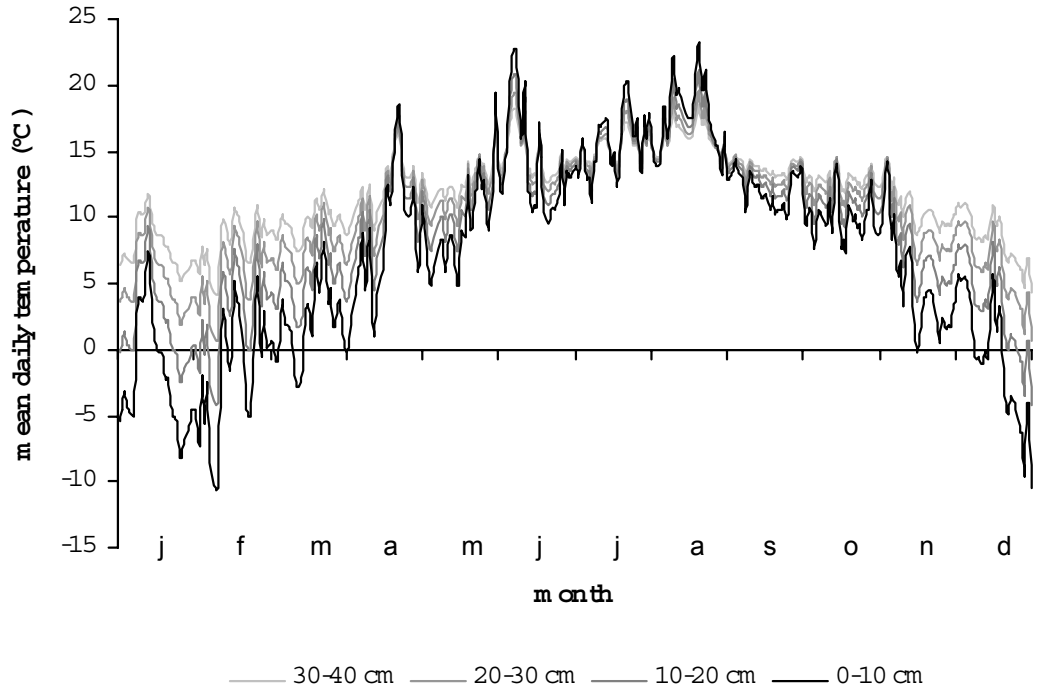


Figure 4.1. Estimated soil temperature at different soil depths in the studied grasslands as derived from the mean daily air temperature (°C) in 1996, which was obtained from the local wether station in Eelde.

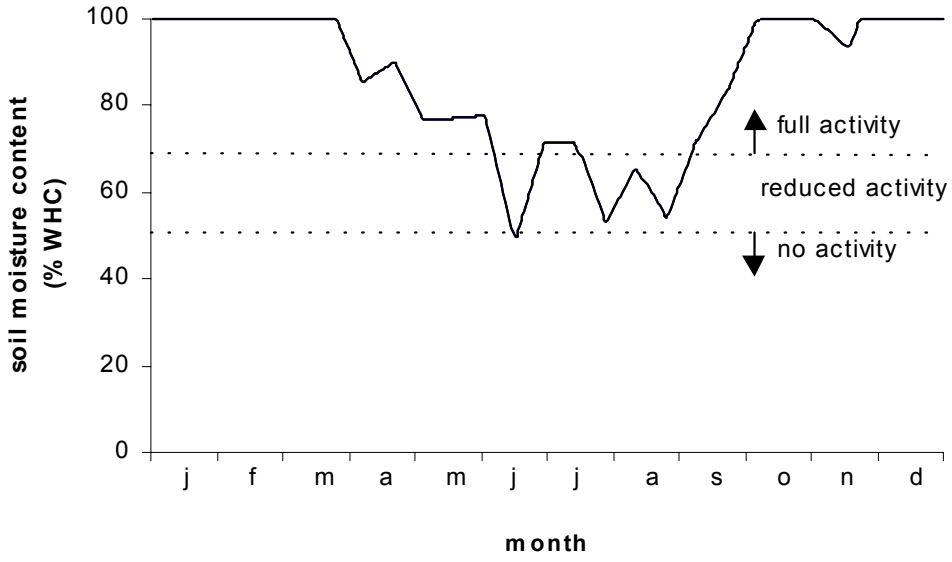


Figure 4.2. Estimated soil moisture content in 1996, expressed as the percentage of the soil water holding capacity (WHC), in the studied grasslands. Critical moisture values (at water potentials of –0.03 and –0.1 MPa) for nematode activity are indicated by dotted lines.

capacity. The dynamics of *m* during the year and the critical *m* values for nematode activity are presented in Fig 4.2. Finally, correction of *R* was obtained by multiplying *R* by the proportion of active nematodes at a certain soil moisture content. Since severe drought conditions are presumably restricted to the upper soil layer, such drought correction was applied only for the 0-10 cm soil and not for the deeper soil layers.

The amount of consumed O_2 was converted to respired CO_2 using a respiration quotient (CO_2 expired/O_2 used) of 0.8 (Sohlenius et al., 1988). The carbon fluxes could then be calculated from the respiration rate data by means of the formule $C = P + R + F$ and $A = P + R$, where *C* is the amount of carbon consumed by nematodes, *A* the amount of carbon assimilated, *P* the amount of carbon converted to nematode biomass and *F* the amount of carbon egested (Duncan and Klekowski, 1975). For the carbon flux calculations the assimilation efficiency *A/C* and production efficiency *P/A* were assumed to be 0.25 and 0.4, respectively (Didden et al., 1994).

Finally, nitrogen fluxes could be estimated by means of the C/N quotients of the nematodes and their food according to Persson (1983). The carbon content is approximately 45%, on a dry weight basis, for nematodes (Jensen, 1984; Borkott, 1989) and 40% for plants. For the calculations, a C/N of 5 was taken for the nematodes (Borkott, 1989; Ferris et al., 1997) and a C/N of 7.5 was assumed for their food, the cytoplasm of plant cells. The efficiency of nitrogen assimilation from the food is likely to be higher than that of carbon. The C/N in faeces was, therefore, assumed to be 1.33 times the C/N of food (Persson, 1983; Didden et al., 1994).

Results

The mean numbers of plant-feeding nematodes, after correction for extraction efficiency and sampling depth, were higher in the high-production field O and the low-production field C than in the other two fields (Table 4.1). However, total and individual biomass of plant-feeding nematodes decreased strongly with time since last fertilisation. As a result of that the total annual respiration rate also decreased. The monthly fluctuations of the CO_2 respiration rate by plant-feeding nematodes in each site are indicated in Fig 4.3.

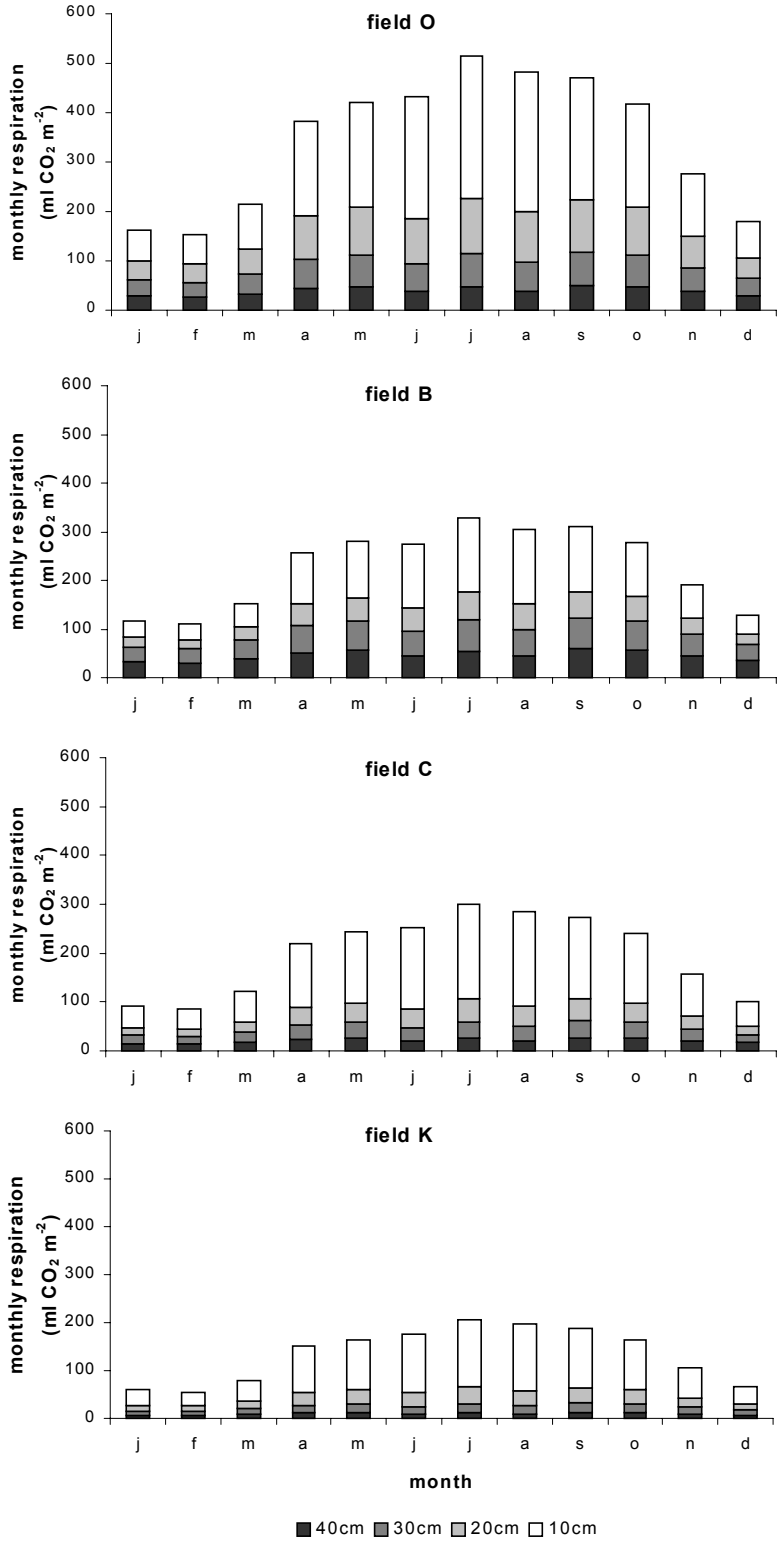


Figure 4.3. Mean monthly CO_2 respiration (ml m^{-2}) by plant-feeding nematodes in different soil layers of four grasslands (O, B, C and K), that had not been fertilised for 6, 10, 23 and 28 years, respectively.

Table 4.1. Numbers, biomass, mean individual biomass and annual oxygen consumption of plant-feeding nematodes in four grasslands (O, B, C and K) that differ in the length of time since fertiliser was last applied.

	Years unfertilised			
	6 (field O)	10 (field B)	23 (field C)	28 (field K)
Numbers (x 10^6 m^{-2})	19.3	13.2	22.6	15.1
Biomass (g dwt m^{-2})	0.20	0.11	0.07	0.05
Mean individual biomass (ng dwt)	10.1	8.5	3.2	3.3
Annual oxygen consumption (l O_2 m^{-2})	5.6	3.7	3.2	2.1

The plant biomass consumed by plant-feeding nematodes in a year decreased with time since last fertilisation from 39.9 g per m^2 in field O to 15.3 g per m^2 in field K (table 4.2). This is approximately 3-5% of the total above- and below-ground standing biomass of the vegetation and 5-9% of the below-ground standing biomass of each site.

Table 4.2. Total biomass consumption and the percentage of root and total standing biomass that is consumed by plant-feeding nematodes in four grasslands that differ in the length of time since fertiliser was last applied.

	Years unfertilised			
	6 (field O)	10 (field B)	23 (field C)	28 (field K)
Consumed plant biomass (g dwt m^{-2} y^{-1})	39.9	26.8	22.6	15.3
total plant biomass (g m^{-2})	912	600	655	396
% total biomass consumed	4.4	4.5	3.5	3.9
root biomass (g m^{-2})	487	326	410	187
% root biomass consumed	8.2	8.2	5.5	8.2

The estimated C and N budgets of the plant-feeding nematode communities in each site are given in Table 4.3. The total annual amount of N mineralised by plant-feeding nematodes ranged from 0.61 g per m^2 in the high-production field O to 0.23 g per m^2 in the low-production field K.

The amount of C allocated to nematode biomass production (Table 4.3) was approximately 20 to 30 times higher than the amount of C in standing nematode biomass per m^2. This is equal to an average nematode life expectancy of 20 days in the high-production field O to 13 days in the low-production fields C and K.

Table 4.3. fluxes of C and N through plant-feeding nematodes and their contribution to total N mineralisation in four grasslands that differ in the length of time since fertiliser was last applied.

	Years unfertilised			
	6 (field O)	10 (field B)	23 (field C)	28 (field K)
Carbon (g C m^{-2} y^{-1})				
Consumption *C*	15.95	10.73	9.06	6.13
Assimilation *A*	3.99	2.68	2.26	1.53
Production *P*	1.60	1.07	0.91	0.61
Respiration *R*	2.39	1.61	1.36	0.92
Defecation *F*	11.96	8.05	6.79	4.60
Nitrogen (g N m^{-2} y^{-1})				
Consumption *C*	2.13	1.43	1.21	0.82
Assimilation *A*	0.93	0.62	0.53	0.36
Production *P*	0.32	0.21	0.18	0.12
Respiration *R*	0.61	0.41	0.35	0.23
Defecation *F*	1.20	0.81	0.68	0.46

Discussion

RELIABILITY OF THE RESULTS

In studies on nutrient fluxes in ecosystems it is of great importance to have an accurate determination of nematode abundance. Approximately 3-5 million plant-feeding nematodes per m^2 were found in the upper 10-cm soil layer of the grasslands studied. These numbers are relatively high compared to most other grassland studies (De Goede and Bongers, 1998). In

most of these studies nematodes were collected in the upper 0-10 cm soil only. In the grasslands studied, however, 30-40% of the total plant-feeding nematode population lived in the 10-40 cm soil layer (chapter 3: Verschoor et al., 2001b). Furthermore, the extraction efficiency of the Oostenbrink elutriator-cottonwool filter method was only 30-45%, due to considerable nematode losses through 45µm sieves and limited movement of nematodes through the cottonwool filter, and vary considerably with nematode type (Verschoor and De Goede, 2000). Such low extraction efficiencies also apply for other extraction methods in which sieves and filters are used. Corrections for sampling depth and extraction efficiency resulted in estimated densities of 13-23 million plant-feeding nematodes per m^2. These results clearly indicate the importance of accurate estimations of the numbers of nematodes to ensure a more reliable estimate of the role of nematodes in C and N fluxes.

The abundance and population structure of plant-feeding nematode species varied during the year (chapter 3: Verschoor et al., 2001b). Nematodes were sampled on only six occasions in 1996. Consequently, it was difficult to distinguish seasonal patterns for the different species. For the calculations, therefore, it was assumed that the nematode density was constant during the year. The variation in the nematode daily respiration rate (without correction for temperature and moisture) between sampling events, however, was relatively low (32.7±4.6, 18.2±4.0, 19.6±4.6 and 13.5±1.0 ml CO_2 day^{-1} m^{-2} in fields O, B, C and K, respectively), which justifies the use of a constant nematode density during the year. Nevertheless, the year-to-year variation in nematode respiration rate can be considerable. At three sampling events in autumn 1995, the numbers of nematodes that were found and the nematode respiration rates that were calculated were approximately twice as high as in autumn 1996.

The mean fresh biomass of individual nematodes ranged between 0.013µg in the lowest-production site and 0.041µg in the highest-production site. These values are considerably lower than in other studies, in which individual weights of approximately 0.1-0.6µg were calculated (see review by Sohlenius, 1980). The low mean individual biomass in the present study can be explained by the high dominance of small nematode species such as *Paratylenchus*, *Pratylenchus* and Tylenchidae. Furthermore, 72% of all nematodes were juveniles, which have a considerably lower biomass than adults. For an adult:juvenile length ratio of 2:1 (Verschoor and De Goede, 2000), the juvenile biomass is on average eight times smaller than that of adults.

For an accurate estimation of the respiration rate of nematode populations it is essential to distinguish between adults, juveniles and species. Frequently, nematode respiration rate has only been calculated on the basis of a mean individual nematode biomass (Brussaard et al., 1990; De Ruiter et al., 1993; Didden et al., 1994). This would generally result, however, in considerable overestimation of the respiration rate. In the present study, such calculations based on mean individual biomass values would result in an overestimation of 20-35% of the actual respiration rate.

Although small species contribute relatively more to the total respiration rate of the nematode community due to a higher metabolic rate (respiration/biomass), low densities of large species may contribute considerably to the absolute amount of respiration. For example, <1% of all plant-feeding nematodes in field O belonged to the genus *Longidorus*, though they contributed up to 20% of the total respiration rate. Unfortunately, female cyst nematodes, which have a biomass (indiv. biomass of approx. 30 μg) considerably higher than even that of *Longidorus* (indiv. biomass of approx. 9 μg), could not be detected by the extraction method that was used. Juveniles of cyst nematodes comprised 1% of the total plant-feeding nematode population in field O. On average 28% of the plant-feeding nematode population consisted of adults. Assuming that 28% of adults also applies for cyst nematode populations, would make the calculated total respiration rate 30-60% higher in the high-production field O. These calculations suggest, therefore, that cyst nematodes, which are important pathogens in agricultural systems, may also have a considerable impact on net primary production and nutrient fluxes in natural ecosystems.

Temperature correction factors derived from Krogh's normal curve (Duncan and Klekowski, 1975), are recommended for a rough estimation of nematode respiration rate (Sohlenius, 1980). The use of mean daily temperature values may result in an underestimation of the total respiration rate due to temperature fluctuations and the exponential relationship between temperature and respiration rate (Sohlenius, 1980). By using the daily minimum and maximum temperature values as night and day temperature, respectively, the calculated respiration rate increased by 18%, which is in fact an overestimation of the total respiration rate. The use of mean daily temperature values, therefore, probably resulted in an underestimation somewhere between 0 and 18%.

The relatively high winter temperature in the deeper soil layers enables nematodes to stay active during the whole year in these layers. Furthermore, they will be less affected by temperature fluctuations. Without correction for depth-dependent temperature fluctuations, however, an underestimation of the total respiration rate of only 2-5% was found.

Very little is known about the relationship between respiration rate and soil moisture content. Storey (1984) found a quadratic relationship between moisture and respiration rate, but this relationship was based on only three soil moisture contents that were all greater than 20%. In the present study, I assumed that nematodes in a loamy sand soil entered anhydrobiosis at –0.03 MPa and reached 100% anhydrobiosis at –0.1 MPa (as indicated by Demeure et al. (1979). I calculated, on the basis of pF curves for loamy sand soils (Wösten et al., 1994), that the lowest moisture content in the studied fields corresponded to a water potential of approximately –0.1 MPa. Applying drought correction to the 0-10 cm soil layer, resulted in a reduction of nematode respiration rate of 7-11%. In other studies and soil types, however, nematodes just entered anhydrobiosis at a water potential of <-0.3 MPa (Blake, 1961; Demeure et al., 1979; Freckman et al., 1987), which suggests that critical drought conditions for nematode activity did not occur in the studied fields. On the other hand, the water potential in the rhizosphere is more variable than in the bulk soil because it strongly decreases during the daytime, when water uptake by plants is high, and increases again to normal levels at night. Such varying drought conditions in the rhizosphere of plants may particularly affect plant-feeding nematodes. Although drought may have had a minor effect on nematode activity in the present study, it is potentially an important constraint of nematode activity and respiration, which needs to be considered in nutrient budget calculations.

The conversion efficiencies *P/A* and *A/C* are sensitive parameter values for the calculations of C and N budgets of nematodes. Conversion efficiencies for nematodes are mostly based on laboratory studies of bacterivorous species (Marchant and Nicholas, 1974; Schiemer et al., 1980; Woombs and Laybourn-Parry, 1985; Herman and Vranken, 1988). Plant-feeding nematodes probably have a lower *A/C* than bacterivores due to the higher C/N quotients of their food. Generally, an *A/C* of 0.25-0.40 and a *P/A* of approximately 0.4 are used for the calculation of C and N fluxes through plant-feeding nematodes (Persson et al., 1980; De Ruiter et al., 1993; Didden et al., 1994). A major problem is that the conversion efficiencies are probably not constant, but dependent on species (Ferris et al., 1997), feeding strategy (Ferris, 1982; Schiemer, 1983), developmental stage (De Soyza, 1973; Duncan and Klekowski, 1975; Schiemer et al., 1980; Woombs and Laybourn-Parry, 1985), food quality (Duncan and Klekowski, 1975; Melakeberhan and Ferris, 1988; Powers and McSorley, 1993), food supply (Marchant and Nicholas, 1974; Sohlenius, 1980; Schiemer et al., 1980; Schiemer, 1982) and temperature (Woombs and Laybourn-Parry, 1985). The nutritive quality of the food in particular can affect the assimilation efficiency. For example, a positive

relationship was found between the *A/C* of the grass bug *Leptopterna dolabrata* and food N content (McNeill and Southwood, 1978). Such a relationship may have important implications for the present study, because the nutritive quality of the food for plant-feeding nematodes seems to decline with decreasing productivity of the grasslands (chapter 2: Verschoor et al., 2001a). Assuming a positive relationship between *A/C* and productivity, plant consumption by nematodes in the low-production sites will be underestimated relative to that in the high-production sites.

The C/N quotient of food is also a sensitive parameter for C and N budget calculations (De Ruiter et al., 1994). Plant roots generally have C/N quotients of approximately 50-60 (Van der Krift, 2000). However, such quotients are not appropriate for sap-feeding herbivores such as nematodes. Unfortunately, to my knowledge no information is available on the C/N quotient of the cytoplasm of root cells. The cytoplasm of plants is assumed to have a higher C/N quotient than that of fungi, which is approximately 3 to 4 (Ingham et al., 1986a). Didden et al. (1994) used a C/N for plant cytoplasm of 10 for their calculations This seems rather high, considering the presence of nitrogen-rich organs and the high concentrations of amino acids in cells. A C/N quotient of 7.5, therefore, seems to be a more realistic estimate.

CONTRIBUTION OF PLANT-FEEDING NEMATODES TO C AND N FLUXES

The amount of N consumed by plant-feeding nematodes, 0.8-2.1 g m^{-2}, was much higher than the values calculated for an arable soil, approximately 0.1-0.4 g m^{-2} (Sohlenius et al., 1988), and a rye field, approximately 0.1 g m^{-2} (Wasilewska, 1974). This is mainly the result of the high, but more accurate, estimations of nematode abundance in the present study. Paustian et al. (1990) estimated the C and N flows in four different cropping systems and found a standing crop biomass of approximately 80% of the gross primary production. Assuming similar relationships between standing crop biomass and gross primary production in the grasslands of the Drentse A nature reserve, approximately 3-4% of the total C uptake and 10-15% of the total N uptake by plants was consumed by plant-feeding nematodes.

Approximately 29% of the consumed N was mineralised by the plant-feeding nematodes. The best available information on total N mineralisation in my study sites is given by Olff et al. (1994). They found a total N mineralisation of 12.4, 17.6, 14.0 and 6.1 g m^{-2} in moist grasslands of the same study area that had not been fertilised for 2, 6, 19 and 45 years respectively. Assuming that the total N mineralisation in the grasslands of the present study is

comparable to these values, plant-feeding nematodes contributed approximately 2-5% to the total N mineralisation.

Although the direct contribution of plant-feeding nematodes to N mineralisation is low, they also indirectly contribute to N mineralisation through the input of organic N to the soil. The calculations showed that as a result of defecation and a high turnover rate of nematode biomass, almost all the consumed N was returned to the soil. Furthermore, root herbivory by nematodes may increase root exudation by leakage of nutrients from damaged root cells (Yeates et al., 1999a). Scott (1979) assumed that some 50% of the total consumption is wasted in this way. Such nematode-induced root exudation has yet to be quantified, but is probably high. For example, Yeates et al. (1999a) found a measurable increase of ^{14}C in the soil compartment after nematode herbivory as early as within 15 days after pulse-labelling of the host plant. The organic N input to the soil by plant-feeding nematodes can be used as a food source for micro-organisms and can subsequently be mineralised by the feeding activities of the microbial-feeding soil community. Consequently, the functional contribution of plant-feeding nematodes to N mineralisation may be considerably more than the estimated 2-5%.

The relative amount of nematode-induced root exudation depends on the size and feeding type of the nematode species. Small migratory ectoparasites generally seem to increase root exudation relatively more so than larger semi-endoparasites (Yeates et al., 1999b). This may have implications for the comparison of C fluxes in the studied grasslands because the proportions of nematode species with different feeding strategies and sizes changed with the time since last fertilisation (chapter 2: Verschoor et al., 2001a).

PLANT BIOMASS CONSUMPTION

Invertebrate herbivores have been reported to consume 1-10% of the total net primary production in grasslands (Curry, 1994). Plant-feeding nematodes have been found to be the major invertebrate herbivores, responsible for 46-67% of the total consumption by all herbivores (Scott et al., 1979). Curry (1994) even referred to a study of Heal and MacLean (1975), in which plant-feeding nematodes contributed up to 90% of the total consumption by all herbivores. In the present study it was estimated that plant-feeding nematodes consumed 3-5% of the total standing biomass, which is within the ranges presented by Curry (1994), and indeed illustrated the large contribution of plant-feeding nematodes to total herbivore consumption.

Nematode consumption in relation to the root standing biomass (5-9%) is relatively high. This is particularly notalble considering that nutrient losses by nematode-induced root exudation were not included in the calculations. These values are, however, not very indicative of the overall impact of root herbivory by nematodes on plant productivity, because herbivory may also reduce plant growth by disordering the physiology of the plant. On the other hand, intermediate levels of (nematode) herbivory may also increase the primary production of grasslands by stimulating plant regrowth (Curry, 1994; Stanton, 1983).

In cases of high levels of herbivory, plant regrowth can be insufficient to overcome the detrimental effects of herbivory. Since the spatial distribution of nematodes is generally clustered, such a situation might occur in specific local hotspots with high nematode densities. For example, I estimated that in hotspots with the highest nematode densities in the Drentse A grasslands up to 43% of the root standing biomass was consumed by plant-feeding nematodes, whereas outside these hotspots only 2-5% of the root standing biomass was consumed. At such hotspots, plant-feeding nematodes might affect the competition between plant species. Eventually, such local effects on plant performance may result in the replacement of plant species.

VERSCHOOR, B.C. DE GOEDE, R.G.M., BRUSSAARD, L. Do plant-feeding nematodes have differential effects on the productivity of an early- and a late-successional plant species? Submitted to *Plant and Soil.*

5. Do plant-feeding nematodes have differential effects on the productivity of an early- and a late-successional plant species?

Abstract We have examined the interaction between plant-feeding nematodes and plant species from different stages of grassland succession. In these grasslands fertiliser application was stopped in order to restore the former nutrient-poor ecosystems. This management resulted in a reversed succession of high- to low-productivity. Plants species of high-production habitats are in general supposed to be more sensitive to nutrient depletion than species of low-production habitats. Furthermore, the sensitivity of plants to herbivory is supposed to be higher at nutrient-poor conditions. We hypothesised, therefore, that at a low nutrient supply rate the growth of an early-successional plant species will be more reduced by nematode herbivory than the growth of a late-successional species. To test this hypothesis, nematodes isolated from an early-successional high-production field and a late-successional low-production field were inoculated to sterilised soil planted with seedlings of either *Lolium perenne* (an early-successional species) or *Festuca rubra* (a late-successional species). The experiment was performed at low and high supply rates of nutrients.

At increased nutrient supply rates, the plant biomass of *L. perenne* increased relatively more than that of *F. rubra*. We found no support for our hypothesis, however, because the inoculated nematodes did not affect the productivity of the two plant species. These results suggest that changes in the nutrient availability rather than plant-feeding nematodes affect plant succession in impoverished grasslands.

On the other hand plant species and nutrient supply rate significantly affected the density and composition of the plant-feeding nematode community. Plant-feeding nematodes in general reproduced better on *L. perenne* than on *F. rubra*. A high nutrient supply resulted in decreased numbers of plant-feeding nematodes, which might have been due to direct toxic

effects of the applied nutrient solution. Our results suggest that the succession of the plant-feeding nematode community is probably more affected by changes in the plant community than the other way round.

Introduction

In certain areas of the Netherlands, agricultural grasslands have been set aside to allow restoration of former species-rich plant communities that had disappeared after enhanced fertilisation. Present day restoration management reduced nutrient supply by the cessation of fertiliser application, while haymaking continued to deplete the fertility status of the soil. As a result of the reduced nutrient availability, plant species characteristic of high-production habitats have been replaced by species that were better adapted to nutrient-poor conditions (Bakker, 1989; Olff and Bakker, 1991).

Root-herbivory by nematodes can be an important factor affecting such plant succession in grasslands (Brussaard et al., 1996; Bardgett et al., 1999b; Olff et al., 2000). Among root-herbivores in grasslands, plant-feeding nematodes are the most abundant group. Despite their small contribution to the total biomass of root-herbivores, nematodes can be important constraints of primary production in grasslands as was indicated by plant biomass increases of 25 to 59% after nematicide treatment (Smolik, 1977; Stanton et al., 1981; Ingham et al., 1986b; Ingham and Detling, 1990). Interactive effects of plant-feeding nematodes with other soil-borne pathogens could also cause such effects of nematodes on the primary production. By their feeding activities, plant-feeding nematodes may create entries in the roots for pathogenic fungi and bacteria and hence increase the vulnerability of the plants to such pathogens (De Rooij-van der Goes, 1995; Van der Putten en Van der Stoel, 1998). Particularly, interactions between plant species may be mediated by the development of host-specific pathogen complexes (Bever, 1994; Van der Putten and Peters, 1997; Mills and Bever, 1998; Holah and Alexander, 1999; Olff et al., 2000). Such pathogen complexes are supposed to cause local degeneration of dominant plant species and their subsequent replacement by other plant species (Van der Putten et al., 1993). Eventually, this might result in an acceleration of plant succession.

Such accelerated plant succession in grasslands under restoration management may also be caused by a synergistic effect of root herbivory and reduced nutrient availabilities. Brussaard

(1998) suggested that under nutrient-poor conditions the impact of nematode herbivory on plant growth is higher than under nutrient-rich conditions. He assumed that fertilisation can offset the damage of plant-feeding nematodes by an enhanced root growth, resulting in a lower number of nematodes per gram root. Furthermore, under nutrient-poor conditions nematodes may cause a stronger reduction of mycorrhizal benefit (Brussaard, 1998). Moreover, an increased nutrient stress can result in a higher sensitivity of plants to herbivores (Mattson, 1980; White, 1984). Eventually, this might contribute to the replacement of these stressed plant species by their successors (Schowalter, 1981).

Plant species from high-production habitats are in general more sensitive to nutrient depletion than species of low-production habitats (Boot and Mensink, 1991; Hunt and Cornelissen, 1997). In the present study, therefore, we tested the hypothesis that under nutrient-limited conditions plant species from high-production habitats will be more sensitive to nematode infestation than species of low-production habitats. Furthermore, we hypothesised that plant species will be more affected by plant-feeding nematodes that are isolated from the same habitat, due to the development of host-specific nematode communities in the field.

To test these hypotheses we conducted a greenhouse experiment in which *Lolium perenne*, which is a dominant plant species in high-productive early successional stages, and *Festuca rubra*, which is a dominant species in low-productive late successional stages, were used as model plants. In this experiment both plant species were grown in the presence or absence of early or late successional nematode communities that were inoculated to sterilised soil at low and high fertiliser supply rates.

Materials and Methods

Collection of nematodes and micro-organism suspensions

In the Drentse A nature reserve, The Netherlands, soil was collected in October 1996 in the upper 20 cm soil from both a high-production (field O) and a low-production (field K) grassland. Detailed site descriptions are given in Verschoor et al. (2001a). The high-production grassland represents an early successional stage, which has not been fertilised since 1989, but was extensively grazed by cattle for five further years. The dominant plant

species in this field were *Lolium perenne*, *Holcus lanatus*, and *Agrostis stolonifera*. The low-production grassland represents a late successional field, which has been neither fertilised nor grazed since 1967. *Festuca rubra*, *Agrostis capillaris* and *Anthoxanthum odoratum* dominated the vegetation of the low-production field. The soil type of both fields was classified as loamy fine sand.

Nematodes were extracted from 50 kg of soil from each field using the Oostenbrink method (Oostenbrink, 1960). These nematodes were used as inoculum in the experiment. Inevitably micro-organisms were also introduced by the nematode suspension. To separate the possible effects of these micro-organisms from those of nematodes, nematode-free micro-organism suspensions were made. Micro-organisms were extracted from roots that had been previously removed from the soil samples. 250 g of roots with adhering soil from both fields was blended separately for 10 s and incubated for 72 hrs on 60 µm sieves placed in a dish filled with tap water. Subsequently, the suspension in the dish was sieved three times using a 10 µm sieve, which effectively removed the nematodes, whereas bacteria and small fungal spores could pass the sieve.

SOIL SAMPLING AND TREATMENT

Approximately 200 kg of soil was collected from the upper 30 cm of soil of a nutrient-poor grassland at the Biological Station in Wijster, The Netherlands. The soil is classified as loamy fine sand. The soil was sieved (1 cm mesh size) to remove the coarse fractions, including roots, and then mixed with a nutrient-poor, white sand from a sandpit near Borger, The Netherlands, on a 2:1 volume basis (soil:sand) to reduce the soil fertility at the start of the experiment. The mixed soil was sterilised by γ-irradiation (average dose of 20 kGy). After γ-irradiation soil moisture content and nutrient concentrations were measured in soil subsamples that were dried for 72 h at 40°C.

Pots (1.5 l, Ø 15.5 cm) were filled with 1300 g of mixed soil and inoculated with sterile water or a nematode and/or micro-organism suspension from either the high- or low-production field. Nematodes and micro-organisms that were inoculated together were always isolated from the same field soil. This resulted in a total of 7 treatments: control, nematodes of high-production field, nematodes of low-production field, micro-organisms of high-production field, micro-organisms of low-production field, nematodes and micro-organisms of high-production field, and nematodes and micro-organisms of low-production field. After

inoculation with the nematodes/micro-organisms, the soil was covered with 200 g soil and moistened by spraying. The nematode inocula contained either 42,000-50,000 nematodes from the high-production field or 19,000-23,000 nematodes from the low-production field per pot. This corresponded to half that of their field density on a soil volume basis.

GROWING AND HARVESTING OF PLANTS

Seeds of *L. perenne* and *F. rubra* were germinated in Petri dishes filled with moist sand in growth chambers at 20°C one week prior to planting. In each pot eight seedlings of *L. perenne* or *F. rubra* were planted. The pots were placed in a greenhouse in a randomised arrangement at an average temperature of 20 ± 2°C. Illumination was supplied to maintain a 12:12 hr light:dark photocycle. The position of the pots was randomly changed once a week to avoid interference by possible local climatic differences. The pots were watered daily by spraying and once a week the pots were set to a soil moisture content of 20% (w/w) by weight. Once a week half the pots received 100 ml of a full-strength nutrient solution (4.67 mM K^+, 8 mM NO_3^-, 0.67 mM $H_2PO_4^-$, 2.33 mM SO_4^{2-}, 3 mM Ca^{2+} and 1.33 mM Mg^{2+} per litre). The remaining pots were unfertilised during the first eight weeks of the experiment and then received 10 ml of the nutrient solution diluted with 90 ml of demineralised water each week. Each treatment was replicated five times. The experiment included 5 (replicates) × 2 (plant species) × 7 (nematode/m.o.) × 2 (fertiliser level) = 140 pots. The experiment was set up as a randomised block design, because the nematodes that were used for the inoculum could not be extracted all at once. It consisted of five blocks of 28 pots, each comprising one replicate per treatment. One block per week was set-up in the period between 31 October – 28 November 1996.

The plants were harvested after 14 weeks. The shoots were removed at the stem base. The remaining clod of soil and roots was divided into quarters. One quarter was used for nematode analyses and the remaining part for root analyses. Soil-free roots and shoots were dried for 72 h at 70°C and weighed. After drying and grinding the samples were digested in sulphuric acid, salicylic acid and 30% hydrogen peroxide (Novozamsky et al., 1988). Total N and P were analysed spectrophotometrically with a continuous flow analyser using samples with known chemical composition as standard.

EXTRACTION AND IDENTIFICATION OF NEMATODES

Nematodes were extracted from the unsterilised treatments using a modified Oostenbrink elutriator (Oostenbrink, 1960) and incubated for 48 hours on a double cottonwool filter (Hygia milac filter). The remaining plant roots in the top sieve of the apparatus were cut into pieces approximately 5 mm long and macerated in a blender for 10 seconds. Endoparasitic nematode species were extracted from the macerated roots using a modified centrifuge flotation technique (Coolen and d'Herde, 1972).

After extraction, the nematodes in 10% of the suspension were counted under a low magnification inverted microscope. The taxa composition of the plant-feeding nematode community from each soil sample was also determined using an inverted microscope (×100-400). Classification of the nematodes in feeding groups was made according to Yeates et al. (1993). Besides plant-feeders, the fungal-feeding nematodes were identified as well. These fungal feeders might affect the productivity of arbuscular mycorrhiza (AM), which we regard as an extension of the plant root system.

DATA ANALYSIS

All plant data were analysed using Analysis of Variance (ANOVA). If necessary, data were logarithm or arcsine transformed in order to obtain homogeneity of variances. For the experiment, which was set up in a randomised complete block design, significant block effects were found. Blocks were, therefore, defined in these analyses as a random factor. A Least Significant Difference test (LSD) was used to determine the statistical difference between groups. To test the effects of fertilisation and plant species on the total number of plant- and fungal-feeding nematodes, a Multivariate Analysis of Variance (MANOVA), which is more powerful than a univariate analysis (see Stevens, 1996), was performed. The treatment effects on the individual nematode taxa were analysed using ANOVA. Prior to the analysis, the nematode numbers were ln(x+1) transformed. All statistical analyses were executed using the statistical package Statistica (version 5.0).

Results

Effects of fertilisation

The mean plant biomass, SBR and the tissue-nutrient concentrations for each treatment are given in table 5.1. A summary of the significant, main and interactive effects of the treatments on the plant parameters is given in table 5.2. A high nutrient supply (FE) significantly increased the root and shoot biomass of *L. perenne* and *F. rubra*. The shoot and root biomass of *L. perenne* increased relatively more than that of *F. rubra* (PL×FE). The SBR of *L. perenne* did not change at a higher nutrient supply, but increased for *F. rubra*. At a high nutrient supply, the N and P concentrations in the shoot tissues of *L. perenne* and *F. rubra* increased, whereas these were slightly decreased in the root-tissues of *L. perenne* and unaffected for *F. rubra*.

Effects of nematodes and micro-organism control suspensions

The presence of nematodes or micro-organisms did not have any significant effects on plant biomass (Table 5.2). Some main effects of nematodes and micro-organisms and interactive effects between micro-organisms, nematodes, fertilisation and plant species, however, were found on the N and P concentrations in the plant tissues.

Nematodes from the low-production field increased the root N concentration in *F. rubra*, but decreased the root N concentration in *L. perenne* (LP-field: NE). Nematodes from the high-production field, generally increased root N at all treatments, except for *L. perenne* in the absence of micro-organisms (HP-field: PL×NE×MO).

Micro-organisms from the high-production field negatively affected the P concentration in the shoots (HP-field: MO). However, no such effect was found at a high nutrient supply when nematodes were present (HP-field: FE×NE×MO). Micro-organisms from the low-production field reduced the P concentration in the roots (LP-field: MO). An interactive effect of nematodes and micro-organisms of the low-production field and fertilisation on the shoot P concentration was found (LP-field: FE×NE×MO). At a low fertiliser supply rate, nematodes without micro-organisms increased shoot P concentration, whereas no effect was found at the other treatments. At a high nutrient supply rate, the shoot P concentration was lower in the presence of nematodes and micro-organisms than in the control without them.

Table 5.1. Mean values of dry biomass, shoot-to-total biomass ratio (SBR) and total N and P tissue content in the presence/absence of nematodes and/or nematode-free micro-organism suspension (m.o) isolated from a high-production (HP) or a low-production (LP) field at a low and high nutrient supply. Statistical differences between means were tested by LSD.

Inoculum HP-field	Low nutrient supply				High nutrient supply			
	-nematodes		+nematodes		-nematodes		+nematodes	
	-m.o.	+m.o.	-m.o.	+m.o.	-m.o.	+m.o.	-m.o.	+m.o.
				Festuca rubra				
Shoot dwt (g)	4.35b	3.96b	3.73b	4.39b	12.17a	12.11a	11.99a	11.12a
Root dwt (g)	6.17b	7.10b	4.83b	5.22b	11.19a	12.45a	11.19a	10.51a
SBR	0.44bc	0.41c	0.45bc	0.48bc	0.52ab	0.52ab	0.55a	0.53ab
N-shoot (mg/g)	5.96d	6.18cd	6.73bcd	6.31cd	8.34a	7.34abc	7.82ab	8.18ab
N-root (mg/g)	4.45	4.20	4.81	4.89	4.41	4.71	5.01	4.45
P-shoot (mg/g)	1.48c	1.46c	1.51bc	1.41c	1.91a	1.73ab	1.90a	1.88a
P-root (mg/g)	0.84	0.79	0.88	0.91	0.88	0.86	0.93	0.82
				Lolium perenne				
Shoot dwt (g)	5.01b	5.22b	5.00b	5.21b	12.74a	13.40a	13.76a	12.25a
Root dwt (g)	4.91b	6.16b	5.32b	5.13b	19.28a	14.86a	20.28a	14.56a
SBR	0.51ab	0.46ab	0.49ab	0.51a	0.40b	0.48ab	0.43ab	0.49ab
N-shoot (mg/g)	6.04b	5.71b	5.75b	6.03b	8.31a	7.95a	7.47a	8.84a
N-root (mg/g)	5.49ab	4.85abc	5.61a	5.70a	3.82cd	4.34bd	3.67d	4.94ad
P-shoot (mg/g)	1.48b	1.32b	1.42b	1.43b	1.79a	1.56ab	1.49b	1.77a
P-root (mg/g)	1.15a	1.01a	1.14a	1.14a	0.76bc	0.79bc	0.71c	0.93ab

Inoculum LP-field	Low nutrient supply				High nutrient supply			
	-nematodes		+nematodes		-nematodes		+nematodes	
	-m.o.	+m.o.	-m.o.	+m.o.	-m.o.	+m.o.	-m.o.	+m.o.
				Festuca rubra				
Shoot dwt (g)	4.35b	4.27b	4.17b	4.05b	12.17a	13.16a	11.88a	12.38a
Root dwt (g)	6.17b	4.81b	4.63b	6.21b	11.19a	13.74a	11.35a	14.33a
SBR	0.44ab	0.49ab	0.49ab	0.42b	0.52a	0.49ab	0.52a	0.50ab
N-shoot (mg/g)	5.96d	6.55bcd	6.33cd	6.09d	8.34a	7.61abc	7.89ab	7.72abc
N-root (mg/g)	4.45	4.77	5.18	4.23	4.41	3.99	4.91	4.35
P-shoot (mg/g)	1.48b	1.46b	1.51b	1.44b	1.91a	1.76a	1.81a	1.79a
P-root (mg/g)	0.84a	0.85ab	0.94ab	0.80ab	0.88a	0.70b	0.91ab	0.76ab
				Lolium perenne				
Shoot dwt (g)	5.01b	5.09b	5.29b	4.97b	12.74a	14.56a	14.63a	14.61a
Root dwt (g)	4.91b	5.62b	6.22b	7.26b	19.28a	22.72a	20.41a	18.03a
SBR	0.51a	0.48a	0.46ab	0.42ab	0.40b	0.41ab	0.42ab	0.46ab
N-shoot (mg/g)	6.04b	6.06b	6.16b	6.08b	8.31a	7.77a	7.62a	7.31ab
N-root (mg/g)	5.49a	5.61a	4.94ab	4.47abc	3.82bc	3.35c	3.38c	3.94bc
P-shoot (mg/g)	1.48b	1.53b	1.60ab	1.46b	1.79a	1.60ab	1.60ab	1.63ab
P-root (mg/g)	1.15a	1.18a	1.01a	0.95ab	0.76bc	0.65c	0.66c	0.76bc

Values with similar letters were not significantly different ($P \leq 0.05$)

Table 5.2. Treatment effects (tested by ANOVA) on dry biomass, shoot-to-total biomass ratio (SBR) and total N and P tissue content of *Lolium perenne* and *Festuca rubra* monocultures. HP = high-production and LP = low-production.

Inoculum HP-field		F values							
Treatment	Df	Root Biomass	Shoot Biomass	Total Biomass	SBR	Shoot N	Root N	Shoot P	Root P
Plant species (PL)	1	1.72	12.1*	4.17	0.10	0.41	0.08	7.80*	1.19
Fertilisation (FE)	1	91.6***	1613***	235***	1.33	112***	3.23	52.1**	7.50
Nematodes (NE)	1	2.14	1.51	2.13	1.77	3.25	4.50	0.18	2.07
Micro-org. (MO)	1	0.31	0.03	0.16	0.35	0.04	0.21	12.5*	0.00
PL × FE	1	10.2*	14.9*	8.33*	12.7*	7.61*	10.69*	7.01	72.2**
PL × NE	1	0.28	0.13	0.24	0.33	1.67	0.06	0.17	0.00
PL × MO	1	3.04	0.01	1.67	4.61	4.32	1.22	0.70	1.13
FE × NE	1	0.10	0.01	0.15	0.15	0.66	0.41	0.01	0.19
FE × MO	1	3.84	4.79	4.92	2.39	1.02	1.59	1.66	0.79
NE × MO	1	0.30	0.01	0.35	0.33	4.10	0.50	6.07	1.60
PL × FE × NE	1	0.44	0.00	0.43	0.75	0.52	0.16	0.58	0.58
PL × FE × MO	1	4.11	0.11	3.09	3.64	2.07	1.55	2.20	1.55
PL × NE × MO	1	1.17	5.45	2.72	0.01	4.84	12.6*	3.24	6.11
FE × NE × MO	1	0.24	2.35	0.11	5.19	4.70	0.14	8.25*	0.00
PL × FE × NE × MO	1	0.27	3.59	0.95	0.01	2.05	1.50	0.65	5.32

Inoculum LP-field		F values							
Treatment		Root Biomass	Shoot Biomass	Total Biomass	SBR	Shoot N	Root N	Shoot P	Root P
Plant species (PL)	1	6.93	46.2**	12.8*	1.39	0.69	0.95	7.52	2.32
Fertilisation (FE)	1	128***	743***	279***	0.01	29.6**	8.05*	36.1**	16.0*
Nematodes (NE)	1	0.33	0.01	0.52	0.23	1.03	0.21	0.09	0.33
Micro-org. (MO)	1	3.13	1.42	4.55	1.57	0.51	5.60	3.57	10.8*
PL × FE	1	66.7**	4.42	18.2*	122***	0.06	18.1*	7.89	24.0**
PL × NE	1	0.87	1.66	1.14	0.00	1.13	14.5*	0.02	4.22
PL × MO	1	0.11	0.11	0.10	0.10	0.06	0.61	0.00	2.82
FE × NE	1	1.62	0.59	1.28	1.56	3.06	2.52	1.71	2.82
FE × MO	1	0.02	1.59	0.29	0.69	0.93	0.00	0.36	0.54
NE × MO	1	0.08	0.90	0.00	0.62	0.24	0.30	0.01	0.04
PL × FE × NE	1	1.52	0.11	1.64	1.48	0.32	0.55	0.10	1.29
PL × FE × MO	1	6.57	0.17	9.67*	3.89	0.82	0.38	0.02	0.90
PL × NE × MO	1	1.36	0.58	1.84	0.64	0.28	0.72	0.00	0.37
FE × NE × MO	1	4.67	0.03	10.4*	2.25	7.83	3.41	22.2**	2.55
PL × FE × NE × MO	1	0.31	0.12	0.12	0.31	0.50	0.15	0.89	0.16

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Treatment effects on nematodes

Generally, the numbers of plant- and fungal-feeding nematodes increased during the experiment, particularly nematodes from the high-production field (Fig. 5.1). Plant species and nutrient supply, however, significantly affected the density and composition of the plant- and fungal-feeding nematode community (Table 5.3). At a high nutrient supply significantly lower numbers of plant-feeding nematode taxa were found than at a low-nutrient-supply. The density of the fungal-feeding *Aphelenchidae* did not differ between the fertiliser treatments. *Pratylenchus* (i.e. a mixture of *P. fallax* and *P. crenatus*), Dolichodoridae (i.e. *Geocenamus nanus* and a few *Tylenchorhynchus dubius*) and *Paratylenchus* (i.e. *P. nanus*) from the high-production field and *Paratylenchus* (i.e. *P. veruculatus*) from the low-production field reproduced better on *L. perenne* than on *F. rubra*.

Table 5.3. Results of a (Multivariate) Analysis of Variance ((M)ANOVA) testing the effects of plant species (SP), nutrient supply rate (FE) and nematode-free micro-organism suspensions (MO) on the composition of the plant- and fungal-feeding nematode communities of a high- and low-production field.

	F values						
	SP	FE	MO	SP×FE	SP×MO	FE×MO	SP×FE×MO
	Nematode fauna of high-production field						
MANOVA results							
Plant-feeders (pf)	8.37***	5.84***	0.46	0.45	0.38	0.50	0.73
Plant+Fungal feeders	9.28***	5.77***	0.45	0.42	0.46	0.29	0.87
ANOVA results							
Pratylenchus (pf)	20.22***	17.81***	0.13	0.12	0.41	0.46	0.09
Helicotylenchus (pf)	3.91	6.84*	0.02	0.22	0.19	0.21	0.00
Dolichodoridae (pf)	13.00**	9.36**	0.36	0.75	0.40	0.44	0.08
Paratylenchus (pf)	37.34***	3.75	2.66	0.76	1.10	0.09	2.75
Tylenchidae (pf/ff)	3.76	6.44*	1.39	0.24	0.28	0.55	0.05
Aphelenchidae (ff)	0.01	1.31	0.05	0.29	0.50	0.21	0.13
	Nematode fauna of low-production field						
MANOVA results							
Plant-feeders (pf)	2.78*	4.01*	0.87	0.79	0.35	0.26	0.17
Plant+Fungal feeders	2.35	3.10*	0.69	0.62	0.50	0.21	0.13
ANOVA results							
Pratylenchus (pf)	1.83	14.06***	0.00	1.40	0.06	0.87	0.01
Paratylenchus (pf)	6.56*	5.99*	0.02	0.05	0.11	0.41	0.09
others (pf)	0.04	2.67	3.09	0.18	1.08	0.00	0.65
Tylenchidae (pf/ff)	0.08	13.73***	0.17	0.82	0.07	0.26	0.07
Aphelenchidae (ff)	0.00	1.03	0.33	0.01	1.58	0.30	0.01

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

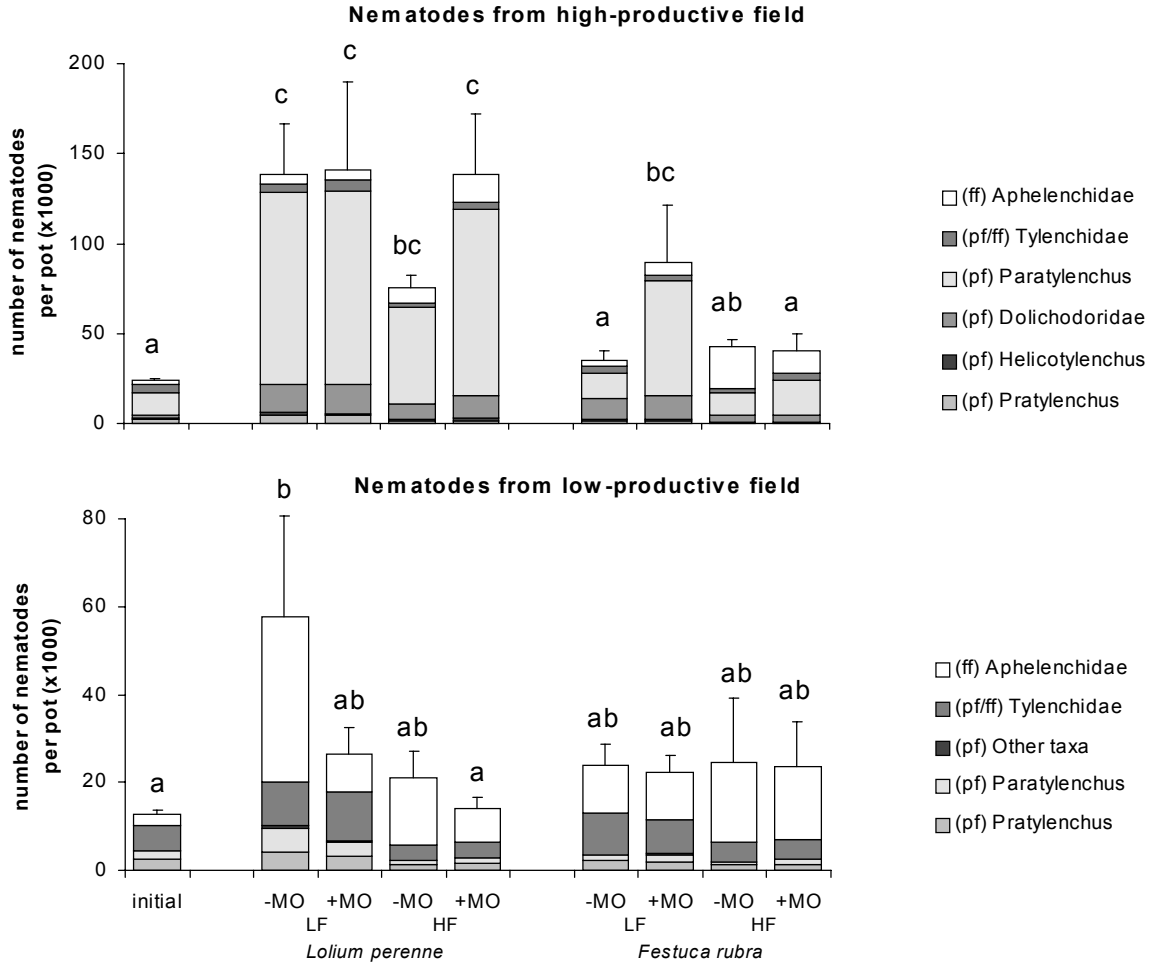


Figure 5.1. Mean (+s.e.) initial and final number of plant-feeding (pf) and fungal-feeding (ff) nematodes, isolated from a high- or low-production field, in pots with *Lolium perenne* and *Festuca rubra* grown in sterilised soil at a low (LF) and high (HF) fertiliser level in the absence/presence of nematode-free micro-organism suspensions (MO). Note the different scale y-axis for fields. Differences in total numbers of plant- and fungal-feeding nematodes were tested by LSD. Values with similar letters were not significantly different ($P \leq 0.05$).

Discussion

EFFECTS OF MICRO-ORGANISMS ON PLANT GROWTH

Inevitably, micro-organisms could have been introduced by the nematode suspensions. To check whether these micro-organisms have unintentionally affected the results, micro-organism suspensions, which were assumed to represent the micro-organism communities in

the nematode suspensions, were added to part of the pots. Fortunately, we found no major effects of the micro-organism suspensions on the plants. We suppose that the concentrations of micro-organisms in the nematode and micro-organism suspensions were relatively low, because most micro-organisms were probably lost during the extraction. A major part of the micro-organisms in the nematode suspensions must have been lost through the 45 μm sieves of the Oostenbrink method (Oostenbrink, 1960). Furthermore, we suppose that in particular the densities of fungal spores were low in the suspensions, because many of the remaining spores after sieving were probably lost on top of the cottonwool filters or 10 μm filters.

EFFECTS OF NEMATODES ON PLANT GROWTH

Estimations of the plant biomass consumption by plant-feeding nematodes in grasslands indicated that in local hotspots nematodes may have a considerable impact on plant productivity (chapter 4). In contrast to these calculations and other studies on nematodes in (semi)natural ecosystems, we found no detrimental effect of nematodes on plant growth. Generally, nematodes are considered as important constraints in primary production in grasslands (reviewed by Stanton, 1988) and may have an important role in the succession of (semi)natural plant communities (reviewed by Mortimer et al., 1999). In most of the studies cited in Stanton (1988) and Mortimer et al. (1999), positive effects on plant growth were found after groups of pathogenic organisms were either killed by the use of (selective) pesticides or by complete sterilisation of the soil. Such methods, however, are often attended by considerable nutrient flushes in the soil. Recently, Troelstra et al. (in press) criticised such methods, and argued that without additional information on the effects of the apparent nutrient flushes it is impossible to distinguish nutrient effects from pathogen effects. The minor effects on plant growth after inoculation of natural field densities of plant-feeding nematodes to sterile soil (this study; Maas et al., 1983; De Rooij-van der Goes, 1995) strengthen the supposition that in many ecosystem studies nematode effects may in fact be ascribed to enhanced nutrient supplies.

The apparent lack of plant response to nematode infestation in the present study, however, might partly be explained by the co-occurrence of microbial-feeding nematodes in the nematode inoculum (55% of the total nematode community in the high-production field and 41% in the low-production field). The negative effects of belowground herbivory, therefore,

could have been offset by the positive impact of beneficial nematodes on the nutrient availability for plant root uptake (Seastedt et al., 1988).

Considering the high vulnerability of seedlings for nematode infestation (Marschner, 1995), we assumed that the numbers of plant-feeding nematodes in the inoculum were sufficiently high for causing detrimental effects on plant growth. Although, the initial numbers of nematodes per gram soil were only about half the average field density of the total nematode community in our study sites, the numbers of nematodes added per unit root was relatively high at the start of the experiment. Furthermore, the nematodes were inoculated in the same soil layer as the seedlings were planted. Calculations of the plant biomass consumption by nematodes, however, showed that strong detrimental effects of plant-feeding nematodes may only be expected at very high densities of plant-feeding nematodes (chapter 4). In agreement with these results, earlier studies have shown that in general only at much higher nematode densities than found in the field, detrimental effects on plant growth could be observed in pot experiments (Maas et al., 1983; De Rooij-van der Goes, 1995). It is possible, therefore, that in spite of high relative numbers of nematodes per gram of seedling roots at the start of the experiment, the absolute numbers of nematodes per gram of soil were too low to cause detrimental effects on plant growth.

Furthermore, positive effects of nematode herbivory at moderate densities at the end of the experiment might have compensated for damage caused by nematodes in the seedling stage. It is generally known that plants can compensate for growth reductions at moderate levels of (nematode) herbivory (Stanton, 1983; Belsky, 1986). Such compensatory growth can even result in an enhanced plant biomass (Hik and Jefferies, 1990; Järemo et al., 1996; Bardgett et al., 1999a,b). Compensatory plant growth might have been caused by an increased nutrient turnover in the soil that resulted from enhanced microbial activity after herbivore-stimulated root exudation (Bardgett et al., 1998; Bardgett et al., 1999a; Denton et al., 1999; Yeates et al., 1999a,b).

Besides indirect positive effects of plant-feeding nematodes on plant growth, they also might have indirect negative effects by increasing the plant sensitivity to other soil-borne pathogens. De Rooij-van der Goes (1995) found that only in the presence of pathogenic fungi, inoculation of relatively low natural densities of nematodes resulted in a growth reduction of plants similar to those found in unsterilised soil compared to sterilised soil. The interaction between nematodes and fungi might be the result of root cell damage caused by nematodes, which facilitates fungi to invade the roots (Mai and Abawi, 1987). Plant-feeding nematodes, even at fairly low densities, therefore, might act as key species that enhance the

sensitivity of plants for soil-borne pathogens (Van der Putten and Van der Stoel, 1998). Although we do not know whether any pathogenic micro-organisms were introduced by the inoculation of nematodes and micro-organism suspensions, we suppose that indirect effects of plant-feeding nematodes via their interaction with other pathogens were negligible in our study.

Effects of fertilisation on nematode numbers

In spite of an increased number of feeding sites at a high nutrient supply due to a larger root system, the numbers of nematodes were lower than at a low nutrient supply. This could have been caused by a lower food quality, a higher resistance of the plant or direct toxic effects of high nutrient concentrations in the soil. A lower food quality could have resulted from a decreased concentration of photosynthates at high nitrogen supplies (Hehl and Mengel, 1972). Nitrogen, however, is generally considered to be the limiting factor for many herbivores (Mattson, 1980; Seastedt, 1985; Yeates, 1987). Since the nitrogen concentration in the roots increased at a higher nutrient supply, an increase rather than a decrease of plant-feeding nematodes could be expected. On the other hand, a reduced nutrient-stress due to a high nutrient supply might lead also to an increased allocation of resources to costly, but effective defensive systems (Rhoades, 1985).

In a field experiment (chapter 7) the numbers of nematodes were reduced five weeks after fertilisation with nutrient solutions, whereas they had increased after 12 weeks when compared to the unfertilised treatment. The latter increase of nematodes suggests that the food conditions for plant-feeding nematodes increased after fertilisation, but that some of the nematodes were inactivated or killed shortly after they were exposed to the nutrient solutions. We suppose, therefore, that the reduced nematode abundance under conditions of high nutrient supply have resulted from a direct toxic effect of the nutrient solution on the nematodes rather than of a reduced food quality or increased resistance.

Specificity of nematodes for host plants

Most nematode taxa, including species of high- and low-production fields, reproduced better on the early-successional *L. perenne* than on the late-successional *F. rubra*. These results are in contrast to our expectations that under each plant species host-specific nematode communities will develop. Also a field survey of plant-feeding nematodes under different

plant species revealed only poor evidence for the presence of host-specific nematode communities in the studied grasslands (chapter 2; Verschoor et al., 2001a). The higher mean densities of all nematode species under *L. perenne* compared to *F. rubra*, suggest an overall higher palatability of *L. perenne*. Plant species from high-production habitats generally have a higher nutrient uptake rate, a higher growth rate, a shorter tissue life span and a lower tissue density than species from nutrient-poor environments (Ryser, 1996a,b). Since the life expectancy of plant tissues is strongly correlated with the palatability of plants (Southwood et al., 1986; Coley, 1988), plant species from low-production habitats may in general have a lower palatability to herbivores. The low palatability of these plant species can result from a lower N-content (Mattson, 1980; Lambers and Poorter, 1992), increased chemical defence (Edwards-Jones and Brown, 1993) or mechanical defence facilitated by a greater tissue density. We found no indications that the higher palatability of *L. perenne* resulted from a higher nitrogen concentration in the roots, since the nutrient concentrations in the roots of *L. perenne* and *F. rubra* were not significantly different. Nutrients, however, can be located in plant tissues or cell structures that are inaccessible for sap-sucking nematodes. Total N, therefore, may not always be a good indicator of available N (Curry, 1994).

Conclusions

Our results indicate that the early-successional species *L. perenne* is more vulnerable to nutrient stress than the later-successional *F. rubra*. Plant-feeding nematodes at half the field density, however, had only minor direct effects on plant growth. We found, therefore, no support for our hypothesis that under nutrient-poor conditions the growth of the early-successional species *L. perenne* was more reduced by nematode herbivory than the growth of the later-successional *F. rubra*.

On the other hand plant species and fertilisation significantly affected the density and composition of the plant-feeding nematode community. Generally, nematodes reproduced better on the early-successional *L. perenne* than on the late-successional *F. rubra*. Fertilisation had a negative effect on the numbers of nematodes.

These results indicate that the succession of plant-feeding nematodes in impoverished grasslands is probably more affected by changes in the vegetation than the other way round.

VERSCHOOR, B.C. PRONK, T.E. DE GOEDE, R.G.M., BRUSSAARD L. Do plant-feeding nematodes affect the competition between grass species during reversed vegetation succession? Submitted to *Journal of Ecology*.

6. Do plant-feeding nematodes affect the competition between grass species during reversed vegetation succession?

Abstract We have examined the effects of plant-feeding nematodes on the competition between the plant species *Holcus lanatus* and *Anthoxanthum odoratum*. In our study area, the Drentse A nature reserve, The Netherlands, *H. lanatus* is representative of a high-production grassland community, which has gradually been replaced by a low-production community, represented by *A. odoratum*, after the application of fertiliser was stopped. Stressed plants are generally considered to be more sensitive to herbivory. We hypothesised, therefore, that the presence of plant-feeding nematodes would add to the reduction of the competition of *H. lanatus* resulting from nutrient limitation, in favour of *A. odoratum*. This hypothesis was tested by the analysis of an adjusted De Wit replacement series in comparison with monocultures. In this experiment soil of a high-production grassland was either treated or untreated with nematicides both at a low and high nutrient supply.

The biomass production of both plant species was negatively affected by intra- and interspecific competition. In mixed cultures *H. lanatus* was a stronger competitor than *A. odoratum*, but *H. lanatus* was also more sensitive to both plant-feeding nematodes and nutrient limitation than *A. odoratum*. Therefore, plant-feeding nematodes and low nutrient availability reduced the competitive suppression of *A. odoratum* by *H. lanatus*. Low nutrient availabilities did not enhance the effect of plant-feeding nematodes on plant growth and competition, indicating an additive and not a synergistic effect of nematodes and nutrient limitation on plant performance.

We conclude that under natural field conditions plant-feeding nematodes may accelerate succession in grasslands after fertilisation has been stopped. We also conclude that in addition to progressing nutrient stress, plant species-specific differences in tolerance to (generalist) plant-feeding nematodes, rather than host specificity of nematodes, determine the plant species replacement during reversed succession in grasslands.

Introduction

Herbivory is generally considered to be a major factor influencing the structure and composition of plant communities either as a type of disturbance, by causing the mortality of individual plants, or by affecting the competitive relationships between coexisting plant species (Huntly, 1991; Hulme, 1996). Since herbivores rarely kill the host plant, it is generally believed that the principal effect of herbivory is the reduction of the competitiveness of grazed individuals rather than to cause outright mortality (Hulme, 1996). Plant competition could be affected by herbivory as a result of selective consumption of plant species, differential impact on resource partitioning and regeneration of competing plant species, and the alteration of the nutrient cycling in the soil (Huntly, 1991; Louda et al., 1990; Bardgett, 1999a,b).

By affecting the competitive relationships between plant species, plant consumption by herbivores or pathogens could be one of the major factors which modifies the direction and rate of succession (Schowalter, 1981; Crawley, 1983; Brown et al., 1987; Van der Putten et al., 1993; Van der Putten and Peters, 1997). It is frequently asserted that the simultaneous exposition of plants to different forms of stress, such as herbivory, competition or disturbance, has a much greater (synergistic) effect on plants than the summed effect of each stress factor separately (see references in Cottam et al., 1986). Particularly, when environmental constraints limit plant response and compensatory regrowth after selective consumption of one competitor, the impact of herbivory on competitive interactions is greatest (Louda et al., 1990). This implies that herbivores and pathogens can speed up species replacement during succession as soon as resources become limited (Schowalter, 1981).

The literature related to herbivore effects on plant competition has mainly focussed on the above-ground herbivores, although recently the interest for the role of below-ground herbivory in structuring plant communities is increasing (Mortimer et al., 1999). Below-ground herbivory is generally regarded as an important constraint of net primary production in grassland ecosystems (Scott et al., 1979; Stanton, 1988; Brown and Gange, 1990). Particularly, plant-feeding nematodes, which are the most abundant group of root-herbivores, are major consumers in grasslands and can make up more than 50% of the total root and crown consumption (Smolik, 1977; Scott et al., 1979; Stanton et al., 1981; Ingham and Detling, 1990). Evidence has been found that selective grazing of plant-feeding nematodes could affect the competitive relationships between crop and weed species (Chen et al., 1995;

Pantone, 1995) and could hamper the establishment of white clover plants in mixed swards with rye grass (Cook et al., 1992). In natural dune ecosystems soil organisms, probably a combination of plant-feeding nematodes and soil-borne fungi, were supposed to affect plant competition (Van der Putten and Peters, 1997).

In the present study the effects of nematodes, nutrient limitation and their interaction on the competitive relationship between a fast-growing, light-competing and a slow-growing, nutrient-competing plant species were investigated. The fast-growing *Holcus lanatus* L. and the relatively slow-growing *Anthoxanthum odoratum* L. were used as model plant species. Both plant species are representatives of different successional stages of permanent grasslands, in which the nutrient availability in the soil is artificially reduced by cessation of fertiliser application while nutrients keep on being removed by hay-making. Such management results in the replacement of fast-growing light-competitors by slow-growing nutrient-competitors (Bakker, 1989; Olff and Bakker, 1991). We hypothesised that at a reduced nutrient availability the stressed fast-growing light-competitors are more vulnerable for plant-feeding nematodes than the unstressed slow-growing nutrient-competitors. The presence of plant-feeding nematodes, therefore, would add to the reduction of the competition by *H. lanatus* resulting from nutrient limitation, in favour of *A. odoratum*.

Material and Methods

SITE DESCRIPTION

In the Drentse A nature reserve, The Netherlands, soil was collected in a grassland site (field O) which was managed according to standard agricultural practice typical for permanent grasslands until 1989 when fertilisation was stopped. From 1989 to 1993 this site was extensively grazed, whereas from 1994 onwards it was used for hay-making to reduce the nutrient availability in the soil. The vegetation in this site was dominated by *Lolium perenne*, *Holcus lanatus, Agrostis stolonifera* and *Ranunculus repens* indicating a high nutrient availability. Long-term studies in the Drentse A nature reserve have shown that when the nutrient availability decreased with time of non-fertilisation the vegetation first becomes dominated by *H. lanatus* and later by slower-growing species which are more efficient

nutrient users, such as *Festuca rubra* and *Anthoxanthum odoratum* (Bakker, 1989; Olff and Bakker, 1991). The soil in field O contained a high density and diversity of plant-feeding nematodes, compared to fields that had not been fertilised for 10 to 28 years (chapter 2: Verschoor et al., 2001a).

SOIL SAMPLING AND SOIL TREATMENT

The soil was collected in October 1998 in the upper 25 cm soil layer of the grassland site. The soil was sieved (1 cm mesh size) to remove roots and coarse material and thoroughly mixed. For a pilot study 20 kg of soil was sterilised by gamma-irradiation (20 kGy). Half the sterilised and half the unsterilised soil were treated with a combination of the nematicides Phenamiphos (Nemacur: 0.25 g per kg fresh soil) and Oxamyl (Vydate: 0.025 ml per kg fresh soil) to eliminate the nematodes. The soil moisture content was measured after drying five subsamples of 1000 g fresh soil for 7 days at 40°C.

COLLECTING AND GROWTH OF PLANTS

In 1997 seeds of *H. lanatus* and *A. odoratum* were collected in the study area. The seeds were germinated in Petri dishes filled with moist sand in the greenhouse one (*H. lanatus*) or two weeks (*A. odoratum*) prior to planting. In November 1998 pots (1.2 l, Ø 12 cm) were filled with 1070 g soil, of which half the pots were filled with nematicide-treated soil and half the pots with untreated soil. After twelve days the seedlings were planted in each pot.

The pots were placed in the greenhouse in a randomised arrangement with a light/dark photocycle of 16/8 hrs and a temperature of 20/16 °C. The position of the pots was randomly changed once a week to avoid the interference of possible local environmental differences. The pots were watered daily by spraying and once a week the pots were set to a soil moisture content of 20% (w/w) by weighing.

During the first four weeks after planting no nutrient solution was supplied. Thereafter, 15, 30 and 50 ml Steiner solution was supplied per pot to half of the pots in weeks 5, 6, and 7-12, respectively, to meet increasing plant requirements. The other half of the pots remained unfertilised for 8 weeks until nutrients became perceptibly deficient for the plants. After 8 weeks these pots received only 5 ml Steiner solution each week to let the plants survive at a minimal nutrient availability.

PILOT STUDY: SIDE EFFECTS OF NEMATICIDES

Nematicides might have a slight phytotoxic effect on plants. On the other hand nematicides could stimulate plant growth as they contain elements such as phosphate that inadvertently might act as a fertiliser. To test these possible side effects on plant growth, 8 plants of *H. lanatus* or *A. odoratum* were planted in monoculture in sterilised soil that was treated or untreated with nematicides with or without supply of fertilisers. Each treatment was replicated 4 times, so that the experiment included 4 (replicates) × 2 (+/-nematicide) × 2 (fertiliser levels) × 2 (plant species) = 32 pots.

REPLACEMENT EXPERIMENT WITH *H. LANATUS* AND *A. ODORATUM*

A replacement series according to De Wit (1960) was applied with 8/0, 6/2, 4/4, 2/6, and 0/8 seedlings of *H. lanatus*/*A. odoratum*. Additionally, both plant species were planted in monocultures of 2, 4, and 6 plants per pot to discriminate between inter- and intraspecific effects. Furthermore, any possible density dependent nematode-effects could be distinguished as the number of (host-specific) nematodes per plant differed between the treatments. Each treatment was replicated 5 times, so that the experiment included 5 (replicates) × 2 (+/-nematicide) × 2 (fertiliser levels) × 11 (plant combinations) = 220 pots.

HARVESTING OF PLANTS

The plants in the pilot experiment and the replacement experiment were harvested after 8 and 12 weeks, respectively. The shoots were cut at the stem bases and dried for 72 hrs at 70°C and then weighed. In the replacement experiment only the root biomass of the monocultures containing 8 plants were measured because in the mixed cultures the root systems of both plant species could not be separated. Total N and P of the roots and shoots of the monocultures containing 8 plants were analysed. After drying and grinding, the samples were digested in sulphuric acid, salicylic acid and 30% hydrogen peroxide (Novozamsky et al., 1988). Total N and P were analysed spectrophotometrically with a continuous flow analyser using samples with known chemical composition as a standard.

EXTRACTION AND IDENTIFICATION OF NEMATODES

Differences in the host-specificity of the plant-feeding nematode species were investigated by examining the nematode communities in the soil of the monocultures containing 6 plants. The nematodes were extracted using a modified Oostenbrink elutriator (Oostenbrink, 1960) and incubated for 26 hours on a double cottonwool filter (Hygia milac filter). The initial number of nematodes was examined in 5 untreated 100 g soil samples before the start of the experiment. The effectivity of the nematicides was checked in soil from 4 nematicide-treated pots at the end of the growth period.

After extraction, 10% of the nematode suspension was counted under a low magnification inverted microscope. The taxa composition of the plant-feeding nematode community in each soil sample was determined under an inverted microscope (100-400x). The classification of nematodes into feeding groups was made according to Yeates et al. (1993). Besides plant-feeders, the fungal-feeding nematodes were identified as well. These fungal feeders might affect the productivity of arbuscular mycorrhiza (AM), which we regard as an extension of the plant root system.

DATA ANALYSIS

All plant data were analysed by Analysis of Variance (ANOVA). In order to meet the assumptions of normality and homogeneity the data were logarithmically, arcsine or square-root transformed if necessary. A Least Significant Difference test (LSD) was used to determine the statistical difference between groups. To test the effects of fertilisation and plant species on the total number of plant- and fungal-feeding nematodes a Multivariate Analysis of Variance (MANOVA), which is more powerful than a univariate analysis (see Stevens, 1996), was performed. The treatment effects on the individual nematode taxa were analysed by ANOVA. Prior to analysis the nematode numbers were ln(x+1) transformed.

Results

EFFECTIVENESS OF NEMATICIDE TREATMENT

The combined application of the nematicides Phenamiphos and Oxamyl effectively reduced the numbers of nematodes at the end of the growth period to 13% of the initial population density ($F_{1,22}$ = 129.95, P < 0.001). Nematicide treatment of sterilised soil did not affect the shoot or root biomass of *A. odoratum* and the shoot biomass of *H. lanatus* (Table 6.1). In the fertilised soil, the root biomass of *H. lanatus* was positively affected by nematicide treatment. However, no stimulating effect of nematicides was found in the unfertilised treatment. Fertilisation generally increased the shoot biomass of both plant species, whereas root biomass was not affected.

Table 6.1. Shoot and root biomass (g) of *Holcus lanatus* and *Anthoxanthum odoratum* monocultures in sterilised soil treated or untreated with fertilisers and/or nematicides together with the *F* values after ANOVA. Within columns letters identify significant differences between group means (LSD, P<0.05).

Data summary

Treatment		*H. lanatus*		*A. odoratum*	
Fertilisation	Nematicide	Shoot biomass	Root biomass	Shoot biomass	Root biomass
-	-	3.83 b	1.64 ab	2.18 b	0.82 a
-	+	4.09 ab	1.74 ab	2.47 ab	0.69 a
+	-	4.43 ab	1.27 b	2.70 a	0.81 a
+	+	4.77 a	2.11 a	2.74 a	0.81 a

ANOVA table

		F values			
		H. lanatus		*A. odoratum*	
Treatment	df	Shoot biomass	Root biomass	Shoot biomass	Root biomass
Fertilisation (FE)	1	6.85*	0.03	12.10**	0.97
Nematicide (NE)	1	1.61	7.21*	2.11	0.86
FE×NE	1	0.02	4.37	1.37	0.29
Error (mean squares)	12	0.0139	0.0177	0.0051	0.0083

P<0.05; ** P<0.01; *** P<0.001

Table 6.2. Biomass, shoot-to-total biomass ratios (SBR), total nitrogen and total phosphorus concentration of *Holcus lanatus* and *Anthoxanthum odoratum* monocultures containing 8 seedlings in soil that was either fertilised (+) or unfertilised (-), both with (+) or without (-) the application of nematicides, and corresponding F values after ANOVA. Within columns letters identify significant differences between group means (P<0.05).

Data summary

Treatment		Biomass (g)		SBR	N_{total} (mg/g)		P_{total} (mg/g)	
Fertilisation	Nematicide	Shoot	Root		Shoot	Root	Shoot	Root
Holcus lanatus								
-	-	2.17 de	1.58 bc	0.58 ab	14.5 d	12.8 bc	2.72 ab	2.07 a
-	+	2.40 cd	3.33 a	0.41 c	13.9 d	10.1 d	2.80 ab	1.76 a
+	-	4.04 b	2.83 a	0.59 ab	22.7 b	13.8 b	2.64 bc	2.07 a
+	+	4.75 a	3.68 a	0.59 ab	21.0 bc	12.6 bc	2.29 c	1.92 a
Anthoxanthum odoratum								
-	-	1.88 f	1.81 b	0.52 b	19.4 c	11.2 cd	2.84 ab	1.89 a
-	+	2.04 ef	1.51 bc	0.58 ab	18.9 c	12.4 bc	3.13 a	2.08 a
+	-	2.63 c	1.31 bc	0.67 a	35.6 a	17.1 a	2.62 bc	2.18 a
+	+	2.44 c	1.15 c	0.68 a	38.5 a	16.8 a	3.03 ab	2.29 a

ANOVA table

		F values						
		Biomass		SBR	N_{total} (mg/g)		P_{total} (mg/g)	
Treatment	df	Shoot	Root		Shoot	Root	Shoot	Root
Plant species (PL)	1	148.5***	34.79***	7.08*	141.8***	11.3**	7.89**	0.06
Fertilisation (FE)	1	247.6***	0.00	15.25**	234.4***	31.2***	5.25*	0.05
Nematicide (NE)	1	5.49*	1.47	2.22	0.06	1.87	0.89	1.21
PL×FE	1	46.4***	7.64**	0.00	22.6***	7.56*	0.59	0.28
PL×NE	1	4.54*	8.38**	7.09*	1.72	4.93*	5.46*	0.01
FE×NE	1	0.78	1.46	2.04	0.29	0.00	0.59	0.69
PL×FE×NE	1	3.59	2.18	4.63*	1.53	1.75	1.78	1.34
Error (mean squares)	30	0.0078	0.1076	0.00078	0.0648	0.0606	0.0086	0.0675

P<0.05; ** P<0.01; *** P<0.001

GROWTH RESPONSE IN THE MONOCULTURES

In the next paragraphs we will describe the effects of fertilisation and nematicide treatment on the plant biomass of *H. lanatus* and *A. odoratum* in monocultures on the basis of the significant ANOVA results presented in Table 6.2.

Fertilisation (FE) resulted in an increased shoot biomass of monocultures of *H. lanatus* and *A. odoratum*, but the fast-growing light-competitor *H. lanatus* produced relatively more extra shoot biomass (overall effect: +92%) than the relatively slow-growing nutrient-competitor *A. odoratum* (+29%) when nutrients were supplied (PL×FE). The root biomass of *H. lanatus* also increased after fertilisation (+32%), whereas the root biomass of *A. odoratum* decreased (-25%). The shoot-to-total biomass ratio (SBR) increased after fertilisation, because relatively more biomass was allocated to the shoots than to the roots when nutrients were supplied. An increased nutrient supply also resulted in an increased N concentration in the shoots and roots of both plant species, whereas the P concentration was not consistently affected.

The application of nematicides resulted on average in a significant increase of the shoot (+15%) and root (+36%) biomass of *H. lanatus*, whereas the shoot and root biomass of *A. odoratum* was unaffected (PL×NE). The SBR of *H. lanatus* decreased in the unfertilised, nematicide-treated soil, but was unaffected in all other treatments. Some small effects of nematicide treatment on the N and P tissue concentrations were found. Nematicide treatment decreased the N content of the roots of *H. lanatus* (particularly in the unfertilised soil), and it increased the P content of shoots of *A. odoratum*.

COMPETITION EXPERIMENT

Monocultures.

In monoculture, *H. lanatus* produced more shoot biomass when nutrients were supplied (Fig 6.1a, Table 6.3: PL×FE). The increase of shoot biomass after fertilisation, however, depended on the number of seedlings that were planted per pot (NR×PL×FE). In the unfertilised treatments the total shoot biomass production of *H. lanatus* did not significantly differ between the plant densities, indicating that the maximum plant yield was reached before the end of the experiment at all plant densities. In the fertilised treatments, however, the total shoot biomass production increased with the number of plants per pot. This indicates that, at least for the lower plant densities, the maximum yield was not reached when fertiliser was applied. The total shoot biomass production of *A. odoratum* increased with plant density in all treatments (Fig 6.1b, Table 6.3). Fertilisation resulted in an increased shoot biomass production of *A. odoratum*, but this was significantly less than the growth response of *H. lanatus*.

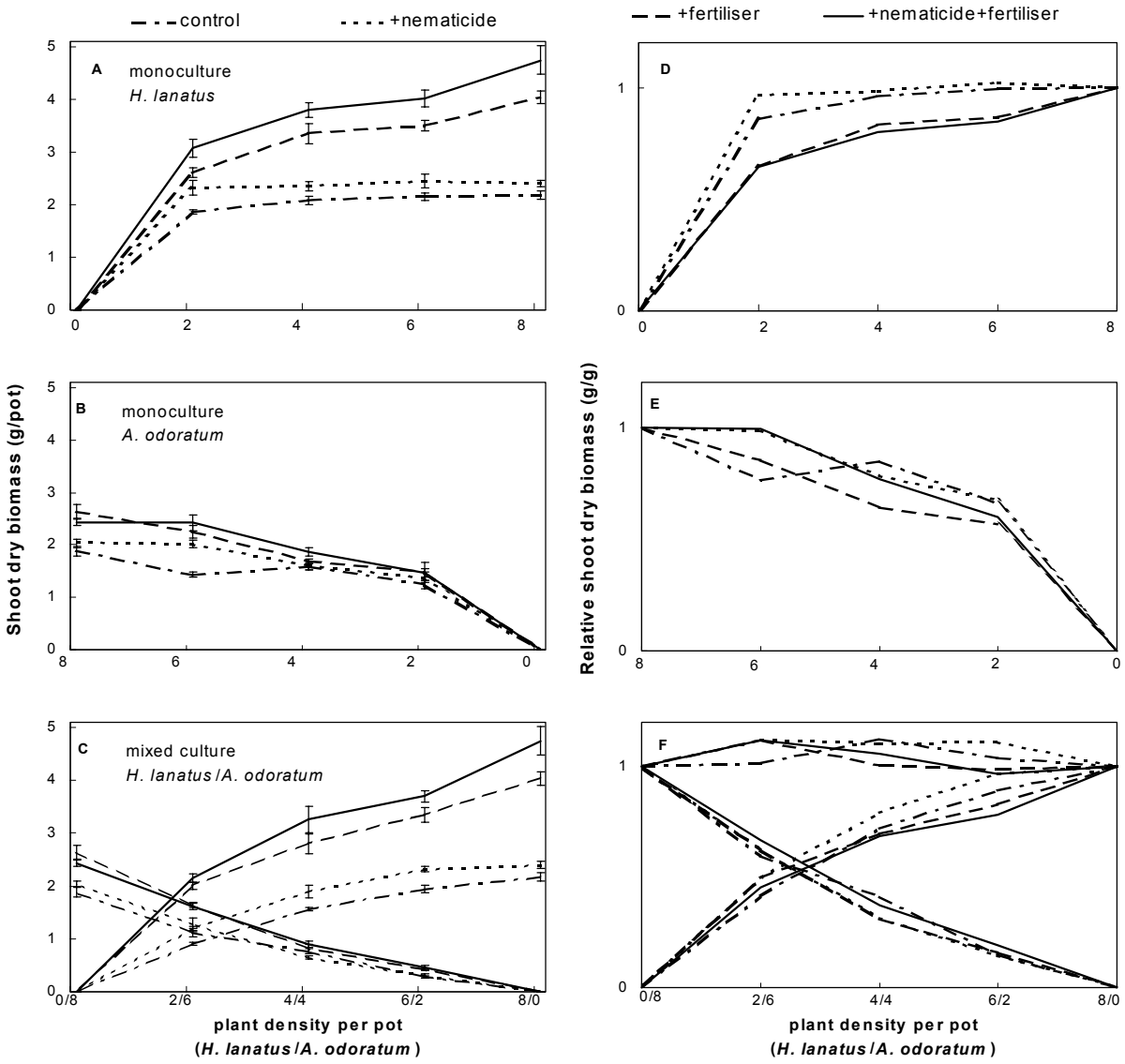


Figure 6.1. Absolute (A-C) and relative (D-F) shoot dry biomass per pot (mean + s.d.) of *Holcus lanatus* and *Anthoxanthum odoratum* when grown in monocultures and in mixed cultures at different plant densities treated or untreated with fertiliser and/or nematicide. The relative shoot biomass is calculated with regard to the total shoot biomass of the monocultures containing eight plants. In F relative yield totals of shoots are shown as well (upper four curves).

Nematicide treatment resulted in an increased shoot biomass of *H. lanatus* at all plant densities (Fig 6.1a, Table 6.3), but did not affect the growth of *A. odoratum* (Fig 6.1b, Table 6.3: PL×NE). A greater effect of nematodes at a low plant density due to a relatively higher initial number of nematodes per plant could not be found. Only in the unfertilised treatment with six plants per pot did nematicide application enhance the growth of *A. odoratum*. For

Table 6.3. Treatment effects (tested by ANOVA) of fertilisation (+/-), nematicides (+/-), and plant numbers (2, 4, 6, and 8 plants per pot) on the total shoot biomass per pot of *Holcus lanatus* and *Anthoxanthum odoratum* in monocultures, mixed cultures, and in mono- and mixed cultures together. In the last column, only the ANOVA results testing the differences between mono- and mixed cultures (*i.e.* the results of interspecific competition) are presented.

Treatment	df	Monocultures	Mixed cultures	Treatment	Mono-Mixed
Mono-Mixed (MM)	1			MM	111.40***
Plant number (NR)	3	81.29***	598.64***	MM×NR	80.71***
Plant species (PL)	1	665.27***	1620.80***	MM×PL	2.49
Fertilisation (FE)	1	410.40***	559.84***	MM×FE	1.57
Nematicide (NE)	1	39.81***	23.36***	MM×NE	10.00***
NR×PL	3	2.96*	37.99***	MM×NR×PL	2.67*
NR×FE	3	14.39***	5.22**	MM×NR×FE	0.41
NR×NE	3	1.12	0.05	MM×NR×NE	1.08
PL×FE	1	102.23***	148.13***	MM×PL×FE	0.74
PL×NE	1	7.09**	16.45***	MM×PL×NE	0.04
FE×NE	1	0.42	0.17	MM×FE×NE	0.58
NR×PL×FE	3	3.78*	1.75	MM×NR×PL×FE	0.87
NR×PL×NE	3	1.63	0.35	MM×NR×PL×NE	0.01
NR×FE×NE	3	0.73	1.03	MM×NR×FE×NE	1.80
PL×FE×NE	1	3.74	0.01	MM×PL×FE×NE	0.25
NR×PL×FE×NE	3	1.24	2.33	MM×NR×PL×FE×NE	3.28*
Error (mean squares)	119	0.00679	0.00614	Error (mean squares)	0.00647

$P<0.05$; ** $P<0.01$; *** $P<0.001$

both plant species the intraspecific competition increased with plant density as is shown by the convex shape of the graphs (Fig 6.1a,b) indicating a decreased shoot biomass per plant.

Mixed cultures.

The shoot biomass production of both plant species was significantly reduced in the presence of its competitor (Fig 6.1c, Table 6.3). Particularly, *A. odoratum* was negatively affected by the presence of *H. lanatus* (MM×(NR)×PL) resulting in a concave shape of the graphs of *A. odoratum*. Shoot biomass of both plant species (but most obviously for *H. lanatus*) was reduced more by the presence of its competitor when it was grown at low densities. Such

significant effects, however, were only found for the unfertilised pots compared to the fertilised pots (MM×NR×FE). Although the presence of nematodes and a low nutrient availability reduced the growth of *H. lanatus* more than that of *A. odoratum*, no evidence was found for a synergistic effect between nematode and nutrient stress on the growth of *H. lanatus*. Neither did we find evidence for a synergistic effect between nematode or nutrient stress and competititive stress. Thus, effects of nematodes, nutrient limitation and competition on the growth of both plant species were completely additive. This is illustrated by the more or less similar graphs for each treatment in Fig 6.1f. In case of synergism these graphs would deviate from each other more pronouncedly.

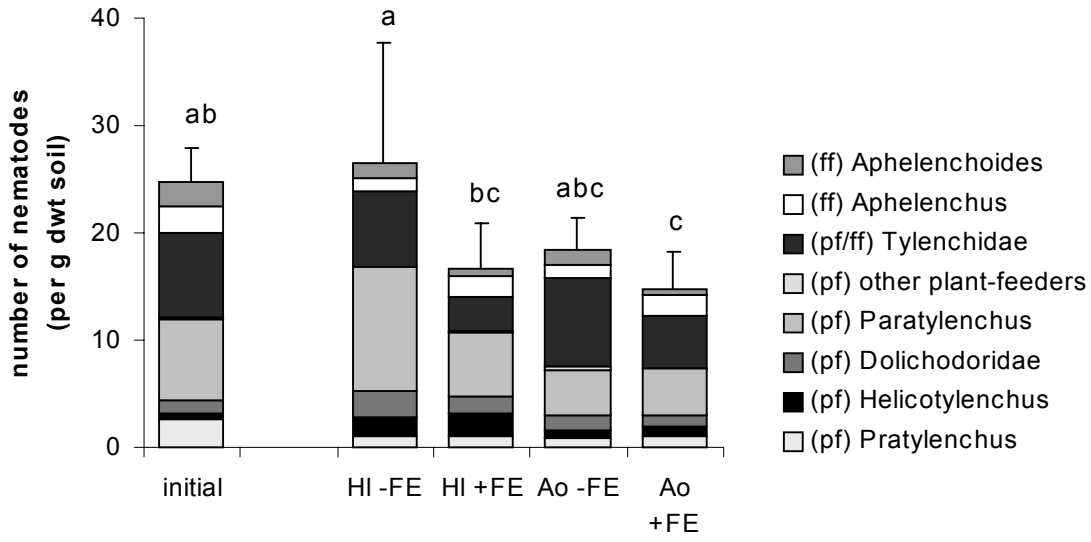


Figure 6.2. Initial and final number (mean + s.d.) of plant-feeding (pf) and fungal-feeding (ff) nematodes in pots with *Holcus lanatus* (Hl) or *Anthoxanthum odoratum* (Ao) grown in fertilised (+FE) or unfertilised (-FE) soil of the nematicide-free treatments.

Characterisation of nematodes.

Plant-feeding and fungal-feeding nematodes were identified to genus level to determine their specificity for *H. lanatus* or *A. odoratum* and their response to fertilisation (Fig 6.2). In general, the total number of plant- and fungal-feeding nematodes at the end of the experiment did not significantly differ from the numbers at the start of the experiment, except for a small reduction in the numbers of nematodes in the fertilised pots grown with *A. odoratum* (Fig 6.2). The MANOVA results, however, showed that the composition of the plant-feeding (+/-

fungal-feeding) nematode community significantly differed between the plant species (Table 6.4). The semi-endoparasite *Helicotylenchus pseudorobustus* and the ectoparasitic Dolichodoridae (mainly *Geocenamus nanus* and some *Tylenchorhynchus dubius*) and *Paratylenchus nanus* reproduced better on *H. lanatus* than on *A. odoratum*. The numbers of the remaining taxa did not differ between the two plant species. Fertilisation resulted in a decrease of the taxa *Paratylenchus*, the plant/fungal-feeding Tylenchidae and the fungal-feeding *Aphelenchoides*, but no significant main effect of fertilisation on the nematode composition was found.

Table 6.4. Results of (Multivariate) Analyses of Variance ((M)ANOVA) testing the effects of different plant species (PL) and nutrient supply (FE) on the composition of the plant- and fungal-feeding nematode community.

	df	*F* values		
		PL	FE	PL × FE
MANOVA results				
Plant- and fungal-feeders (pf+ff)	8	3.95 *	2.73	0.67
Plant-feeders (pf)	6	6.12 **	2.16	1.00
ANOVA results				
Pratylenchus (pf)	1	1.51	0.00	0.00
Helicotylenchus (pf)	1	16.06 **	1.69	0.06
Dolichodoridae (pf)	1	3.63	0.40	0.09
Paratylenchus (pf)	1	20.96 ***	4.89 *	5.82 *
Rare species (pf)	1	1.57	0.17	0.31
Tylenchidae (pf/ff)	1	2.67	7.49 *	0.01
Aphelenchus (ff)	1	0.08	3.70	0.00
Aphelenchoides (ff)	1	0.22	5.74 *	0.13

P<0.05; ** *P*<0.01; *** *P*<0.001; pf = plant-feeder; ff = fungal-feeder

Discussion

Application of the nematicide compounds phenamiphos and oxamyl effectively reduced nematode populations in the field (Kimpinski et al., 1982; Lucas, 1982; Yeates, 1985). Both nematicides were found to have a considerable specificity to nematodes, although some small (indirect) effects on non-target organisms have been observed (Ramsay, 1984; Ross et al., 1984; Hofman, 1988; Zoon, 1995). We applied a combination of both nematicides at the recommended dosages in our experiments, because this combination was the most efficient in

reducing the numbers of nematodes (unpublished data). Furthermore, application of this nematicide combination did not directly affect plant growth in sterilised soil. *H. lanatus* produced relatively more root biomass after nematicide treatment only in the fertilised treatment. Since such a stimulating effect of nematicides was not found in the unfertilised treatment, we are inclined to ascribe this result to chance. Thus, the elimination of plant-feeding nematodes seemed to be the main cause of the increased plant growth after nematicide treatment in unsterilised soil.

The use of De Wit replacement series has been frequently criticised because of its inability to determine density-dependent interactions (Inouye and Schaffer, 1981, Firbank and Watkinson, 1990). Inter- and intraspecific effects could, therefore, not be discriminated. The replacement series is, however, valuable for comparing the outcome of competition between two plant species under different conditions (Firbank and Watkinson, 1990). By studying monocultures at different plant densities, we were able to meet the shortcomings of the replacement series design. The adjusted design made it possible to determine whether there were density-dependent effects of the relatively higher initial number of nematodes per (host) plant at low plant densities. A greater effect of nematodes, however, could not be found at a low plant density. In the case of *A. odoratum*, only in the unfertilised treatment with six plants per pot did the nematicide treatment significantly enhance plant growth. Since no such result was found in the fertilised treatment and it could not be related to a higher density of nematodes per plant, we are inclined to ascribe this result to chance.

The growth of both *H. lanatus* and *A. odoratum* was affected by intraspecific competition. As a result of a faster nutrient depletion the biomass per plant decreased at a higher plant density. An equal total shoot biomass produced in the unfertilised treatment of *H. lanatus* at all plant densities, indicated that the maximum yield was reached at all plant densities before the end of the experiment. In mixed culture, both plant species were negatively affected by the presence of the other species. In all treatments *H. lanatus* was the better competitor for the limited amount of available resources.

In line with our hypothesis, the biomass of the fast-growing, early-successional *H. lanatus* was significantly reduced in the presence of nematodes, whereas the relatively slow-growing, late-successional *A. odoratum* was unaffected by the presence of nematodes. Furthermore, the numbers of plant-feeding nematodes in the root zone of the fast-growing, early-successional species were generally higher than those in the root zone of the slow-growing, late-successional species (this study; chapter 5). In both of these studies, nematode species were

found to reproduce better on the fast-growing, early-successional plant species than on the slower-growing, late-successional species, whereas no nematode species were found that reproduced better on the latter plant species. These results suggest that fast-growing plant species are better host plants for plant-feeding nematodes. In a choice experiment, wireworm species that were captured in our field sites also preferred the fast-growing plant species *L. perenne* and *H. lanatus* to the relatively slow-growing plant species *F. rubra* and *A. odoratum* (Hemerik and Gort, unpublished data). Fast-growing plant species have generally been found to have a higher N-content (Mattson, 1980; Lambers and Poorter, 1992), and a lower chemical and mechanical defense (Edwards-Jones and Brown, 1993; Ryser, 1996a,b), which result in a higher palatability of their tissues (Southwood et al., 1986; Coley, 1988). Our results suggest, therefore, that fast-growing plant species are more attractive host plants for plant-feeding nematodes than slow-growing plant species because of their higher palatability. On the other hand we found a higher total N and P concentration in the tissues of *A. odoratum* compared to those of *H. lanatus* (table 6.2), which does not agree with our expectations. Generally, the numbers of plant-feeding nematodes are considered to be postively correlated to the total N concentration in the plant (Yeates, 1987). However, nutrients can be located in plant tissues or cell structures that are inaccessible for sap-sucking nematodes. Total N, therefore, may not always be a good indicator of available N (Curry, 1994).

Under nutrient-rich conditions, plant species with a high growth rate and a high specific leaf area, such as *H. lanatus*, are better competitors for the limited availability of light than relatively slow-growing plant species, such as *A. odoratum*, (Grime and Hunt, 1975; Lambers and Dijkstra, 1987; Poorter and Remkes, 1990; Olff, 1992b). In spite of their greater palatability, such fast-growing plant species might be able to tolerate high levels of herbivory due to their faster growth rate which may compensate for the herbivore damage (Southwood et al., 1986). On the other hand, fast-growing plant species are more sensitive to nutrient limitation than slow-growing, but efficient nutrient-utilising plant species (Boot and Mensink, 1991; Hunt and Cornelissen, 1997). In our study the fast-growing *H. lanatus* produced approximately 48% less biomass in the unfertilised treatment compared to the fertilised treatment, whereas the biomass of *A. odoratum* was only reduced by about 22% when no fertilisation was applied. The differential response of both plant species to nutrient limitation also resulted in a reduced competitive pressure of *H. lanatus* on *A. odoratum* under nutrient limited conditions, although the growth of *A. odoratum* in the unfertilised treatments was still suppressed in the presence of *H. lanatus*.

Plants that suffer from nutrient stress are supposed to be more sensitive to herbivory than unstressed plants (Mattson, 1980; White, 1984; Louda et al., 1990; Marschner, 1995). Such lower plasticity of plants to herbivory in nutrient-limited conditions can result in enhanced competition between two plant species (Van der Putten and Peters, 1997). We found no evidence, however, that the nutrient-sensitive *H. lanatus* was relatively more sensitive to plant-feeding nematodes than *A. odoratum* when no fertiliser was supplied, although *H. lanatus* tended to produce relatively less biomass than *A. odoratum* in such conditions. Furthermore, combined nutrient and nematode stress did not affect the competition between *H. lanatus* and *A. odoratum* more than the summed effect of both stresses separately. Apparently, nutrient limitation and plant-feeding nematodes had an additive rather than a synergistic effect on the growth and competitive abilities of *H. lanatus* and *A. odoratum*.

Although the reduction of biomass in the presence of nematodes was relatively low (on average 13% for *H. lanatus* and 7% for *A. odoratum*), we suggest that on a local scale in the field much higher growth reductions may occur. The nematode densities in our experiment represented the average density of plant-feeding nematodes in our field site, however a high spatial heterogeneity in the distribution of nematodes exists (chapter 2: Verschoor et al., 2001a). In patches with high densities of plant-feeding nematodes, the effects of nematodes on plant growth may, therefore, be considerable (chapter 4), and this might contribute to small-scale shifting mosaics in grassland vegetation (Olff et al., 2000; Blomqvist et al., 2000). In our study area, the Drentse A nature reserve, the availability of nutrients was gradually reduced with time of non-fertilisation. Small-scale shifts in the vegetation composition are, therefore, likely to result in a gradual replacement of fast-growing plant species characteristic of nutrient-rich conditions by slow-growing plant species characteristic of nutrient-low conditions.

Below-ground herbivory is generally regarded to speed up plant succession (Brown and Gange, 1992; Van der Putten et al., 1993; Van der Veen, 2000). In coastal dune ecosystems the primary succession seems to be particularly driven by the development of species-specific pathogen complexes that affect the growth of the dominant plant species, but not that of the succeeding plant species (Van der Putten et al., 1993; Van der Putten and Peters, 1997). In the grasslands of the Drentse A nature reserve the successional processes are more or less reversed to primary succession, in terms of nutrient availability. Although a nematode survey under different plant species within these grasslands showed some small differences in the composition of the plant-feeding nematode communities between the plant species, clear-cut evidence for species-specific interactions between plants and nematodes had not been found

(chapter 2: Verschoor et al., 2001a). Furthermore, successional changes in the nematode communities seemed to be more affected by qualitative changes within plant species, than by alterations in the species composition of the plant community (chapter 2: Verschoor et al., 2001a). Additionally, no species-specific interactions were found in a greenhouse experiment (chapter 5). We conclude, therefore, that in addition to progressing nutrient stress, species-specific differences in tolerance to (generalist) plant-feeding nematodes, rather than host specificity, may determine the plant species replacement during reversed succession in grasslands.

VERSCHOOR, B.C. DE GOEDE, R.G.M., BRUSSAARD, L. Plant-nematode interactions in a reversed succession of high to low-production grasslands: how plants affect nematodes, but do nematodes affect plants? Submitted to *Oecologia.*

7. Plant-nematode interactions in a reversed succession of high to low-production grasslands: how plants affect nematodes, but do nematodes affect plants?

Abstract In the present study we have investigated the interactions between plants and plant-feeding nematodes in a productivity gradient of four semi-natural grasslands that were unfertilised since 1989, 1985, 1972 and 1967, respectively. Assuming that stressed plants are more sensitive to nematode infestation, we hypothesised that plant species of high-production habitats would be more sensitive to nematode infestation under nutrient-limited conditions than plant species of low-production habitats, whereas they could tolerate high nematode infestations at nutrient-rich conditions. This hypothesis was tested in a factorial field experiment in which the nematode density, nutrient availability and soil pH were manipulated by the application of nematicide, NPK fertilisers and lime, respectively. Furthermore, effects of these treatments on plant productivity were investigated on four grass species that were experimentally introduced to the fields. Fertilisation and liming considerably increased plant biomass in the low-production fields and affected the root nutrient concentrations of the plants. The density of plant- and fungal-feeding nematodes was strongly affected by all treatments, and is suggested to be positively related to the concentration of available nitrogen in the roots. Nematicide treatment had no main effects on vegetation biomass, although it reduced plant-feeding nematode numbers by 70-85%. Nematicide treatment only increased plant biomass in fertilised plots of a low-production field (+72%). In this field, fertilisation considerably increased the numbers of plant-feeding nematodes, which apparently resulted in a strong reduction of plant growth by these nematodes. Furthermore, the average root biomass of *Festuca rubra* when grown in a low-production field increased (+67%) after nematicide application. We concluded, therefore, that in contrast to our hypothesis no

evidence was found that plant species of high-production habitats were more sensitive to nematode infestation.

Introduction

In the past two decades, there is an increasing interest for the role of belowground grazers and pathogens in spatio-temporal vegetation processes. Studies on this subject have been comprehensively reviewed recently (Mortimer et al., 1999; Van der Putten, in press). Particularly, plant-feeding nematodes are considered as major belowground herbivores affecting vegetation composition (Stanton, 1988; Mortimer et al., 1999). In permanent pastures, for example, plant-feeding nematodes inhibited the establishment and persistence of white clover, *Trifolium repens* (Cook et al., 1992). In coastal sand dunes, plant-feeding nematodes, as components of soil pathogen complexes, were presumably involved in the degeneration of dominant plant species, such as *Ammophila arenaria* (Marram grass) and *Hippophaë rhamnoides* (Sea buckthorn) (see references in Van der Putten, in press). Furthermore, such specific pathogen complexes affected the competitive abilities of host plants (Van der Putten and Peters, 1997) and may subsequently contribute to vegetation succession (Van der Putten et al., 1993). Other examples of plant-feeding nematode effects on vegetation composition are given by Olff et al. (2000) and Blomqvist et al. (2000) in grassland ecosystems, who found that local shifts in vegetation patterns could be related to differential sensitivity of coexisting plant species to soil-borne pathogens.

In Dutch grasslands that were formerly managed according to standard agricultural practice typical for permanent grasslands, the application of fertilisers was stopped while hay-making continued to restore the former species-rich plant communities of nutrient-poor grasslands. Although the reduction of nutrient input is considered to be the main driving force of succession, Brussaard et al. (1996) suggested that also root herbivores, such as plant-feeding nematodes, can have an important impact on such reversed succession in grasslands. Estimates of plant biomass consumption by plant-feeding nematodes based on their densities within such grasslands under restoration management, suggested that nematodes can have a considerable impact on the plant productivity (chapter 4). Furthermore, a greenhouse experiment indicated that plant-feeding nematodes can affect the competition between an early- and late-successional plant species in favour of the latter (chapter 6).

In the present study we have investigated the interactions between plants and plant-feeding nematodes during reversed succession by means of a field experiment. We hypothesised that under nutrient-poor conditions, plant species of high-production habitats will be more affected by nematode infestation than plant species of low-production habitats. Hence, suppression of early-successional plant species by late-successional species can be caused by two mechanisms. Firstly, early-successional plant species are more sensitive to nutrient-poor conditions than late-successional species. A high nutrient availability in general results in high numbers of plant-feeding nematodes (Yeates, 1987). Brussaard (1998) suggested, however, that a high nutrient availability may offset damage caused by nematodes because a high root biomass production will result in a low nematode density per gram root. Furthermore, under nutrient-poor conditions nematodes can cause a stronger reduction of mycorrhizal benefit (Brussaard, 1998). Moreover, stressed plants are considered to be more attractive to herbivores, due to an increased concentration of soluble nitrogen in the tissues (White, 1984). A reduced nutrient supply, therefore, could result in a higher sensitivity of stressed early-successional plant species for plant-feeding nematodes. Secondly, species-specific nematode communities can develop under plant species, which may affect the growth of these plant species, but not that of their successors (Van der Putten et al., 1993).

This hypothesis were tested by the application of nematicide at a low and high nutrient availability in four grasslands of different productivity. Since the nutrient availability and net primary production of the grasslands were negatively correlated with the acidity of the soil, we have also examined the effects of liming on the nematode-plant interactions. The study consisted of two experiments: a) a field manipulation experiment in which undisturbed field plots were treated with either nematicide, fertiliser, lime, or combinations of these treatments, and b) a tube planting experiment in which four grass species were grown in plastic tubes, which were inserted in the experimental plots of the most productive and least productive field site. The tubes were filled with soil of the corresponding field and were exposed to one of the fore-mentioned treatments.

Material and Methods

STUDY AREA

The field experiment was carried out in 1997 and 1998 in four semi-natural grasslands of the Drentse A nature reserve, The Netherlands. These grasslands had not been fertilised since 1989 (field O), 1985 (field B), 1972 (field C) and 1967 (field K), and represented different stages of reversed succession ranging from high plant productivity to low plant productivity. During this succession not only the nutrient availability, but also soil pH decreased. A detailed description of the field history and management, soil characteristics, and composition of the plant and plant-feeding nematode communities within these study sites is given in chapter 2 (Verschoor et al., 2001a).

FIELD MANIPULATION EXPERIMENT

experimental set-up

We set up a full factorial experiment with in each field three replicate plots consisting of eight quadrates of 1 m^2. Eight different treatments were established, one for each quadrate: control (C), nematicide (N), fertilisation (F), liming (L), nematicide and fertilisation (NF), nematicide and liming (NL), fertilisation and liming (FL), and nematicide, fertilisation and liming (NFL).

soil treatment

On April 3, 1997 the plots were treated. For the nematicide treatment per quadrate 10 g of the granular nematicide Nemacur (Phenamiphos) was supplied. The nematicide was brought into the soil by dividing it equally over a hundred pits (20 mm ∅) of approximately 3 cm deep. Pits were created also in the untreated quadrates to exclude possible side effects of the soil perforation. A nematode sampling in May 1997 showed no effect of the nematicide on the numbers of nematodes. Therefore, the N, NF, NL and NFL quadrates were retreated with the fluid nematicide Vydate (Oxamyl) on May 30 and June 13. Per quadrate 1 ml Vydate, dissolved in 0.5 l water, was supplied. Before treatment all quadrates (including C, F, L and FL) were mown for a good infiltration of the nematicide.

NPK fertiliser was applied as 25 g N m^{-2} (as NH_4NO_3), 12.5 g P m^{-2} (as NaH_2PO_4) and 25 g K m^{-2} (as KCl). These nutrients were supplied in two portions on April 3 and April 29, dissolved in demineralised water, using 1 l per quadrate. Lime was supplied as 300 g Ca m^{-2} (as $CaCO_3$). After initialising the treatments all quadrates were sprayed with water for a good infiltration of nematicide, nutrients and lime into the soil. Since the plots were mown in May before the retreatment of nematicide, fertilisation (with the same amounts of fertiliser), but not liming, was repeated on May 30 and June 13.

In September 1997 the plots were mown corresponding to the standard management of these grasslands in the Drentse A nature reserve. In March 12 and April 2, 1998 the plots were retreated with the same amounts of Vydate, fertilisers and lime.

nematode sampling and harvest

In October 1997 a bulked soil sample, consisting of nine cores of 14 mm diameter and 10 cm depth, was taken in each quadrate for nematode analyses.

The field manipulation experiment was harvested in July 1998. The plant species composition of each quadrate was estimated according to the Braun-Blanquet method. Thereafter, the vegetation in the centre of each quadrate (in a circle of 57 cm diameter) was harvested. Furthermore, in each quadrate, a root sample, consisting of two cores of 8 cm diameter and 10 cm depth, was taken. The roots were removed from the soil by sieving and washing. The cleaned root and shoot material was dried at 70 °C for biomass estimation. The pH($CaCl_2$) in the soil was determined with a standard glass electrode.

TUBE PLANTING EXPERIMENT

experimental set-up and soil treatment

In March 1997, soil cores of 8 cm diameter and 20 cm deep were taken near the corners of each experimental quadrate of field O and field K (see field manipulation experiment). The collected soil was sieved (1 cm mesh size) to remove roots and coarse material and mixed thoroughly. The soil of each field was divided in eight portions of about 12 kg fresh weight each. Each soil portion was treated according to one of the eight treatments mentioned in the field manipulation experiment. The supplies of nematicide, lime and nutrients, expressed per m^{-2} of soil, were similar to the supplies in the field manipulation experiment. The nematicide Nemacur (60 mg per kg soil) and lime (1500 mg per kg soil) were mixed through the soil. The nutrient solution (10 ml per tube; 10 g l^{-1} N, 5 g l^{-1} P and 10 g l^{-1} K) was supplied

afterwards. Plastic tubes, 8 cm diameter and 20 cm long, were covered with a gauze (10 µm mesh size) at one side and filled with 1 kg of (treated) soil.

Seeds of four dominant grass species in our study sites, *Lolium perenne* L., *Holcus lanatus* L., *Festuca rubra* L. and *Anthoxanthum odoratum* L., were collected in the study site and germinated in climate chambers for two weeks on moist sand. In each tube six seedlings of one plant species were planted and incubated for three weeks in a greenhouse. In April 1997 the tubes were placed in the soil near the corners of each quadrate with one plant species in each corner. The soil treatments in the tubes corresponded with the treatment of the quadrate in which they were placed. In total there were 2 (fields) × 3 (replicate plots) × 8 (treatments) × 4 (plant species) = 192 tubes.

In June 1997 the plants in the tubes were cut and the soil retreated with nutrient solution and the nematicide Vydate, simultaneously with the field plots (see field manipulation experiment).

Harvest

In September 1997 the tubes with plants were removed and taken to the laboratory. The shoots were cut at the stem base. The soil, including plant roots, was removed from the tube and was cut into four parts of about equal size. One quarter of the soil was used for nematode extraction. The other three-quarter of the soil was sieved to collect the roots. The roots and shoots were dried for 72 h at 70 °C and weighed. After drying and grinding the samples were digested in sulphuric acid, salicylic acid and 30% hydrogen peroxide (Novozamsky et al., 1988). Total N and P were analysed spectrophotometrically with a continuous flow analyser using samples with known chemical composition as standard.

EXTRACTION AND IDENTIFICATION OF NEMATODES

Nematodes were extracted from the soil using a modified Oostenbrink elutriator (Oostenbrink, 1960) and incubated for 48 hours on a double cottonwool filter (Hygia milac filter). The remaining plant roots in the top sieve of the apparatus were cut into pieces approximately 5 mm long and macerated in a blender for 10 seconds. After that, the roots were added to the nematode suspension on the cottonwool filter.

After extraction, the nematodes in 10% of the suspension were counted under a low magnification inverted microscope. The species composition of the plant-feeding nematode

community from each soil sample was determined. Classification of the nematodes in feeding groups was made according to Yeates et al. (1993). Besides plant-feeders, the fungal-feeding nematodes were identified as well. These fungal feeders might affect the productivity of arbuscular mycorrhiza (AM), which we regard as an extension of the plant root system.

STATISTICAL ANALYSES

All plant data were analysed using Analysis of Variance (ANOVA). If necessary, data were logarithm or arcsine transformed in order to obtain homogeneity of variances. As the experiments were set up in a randomised complete block design, blocks were defined in these analyses as a random factor. Since we could not collect enough plant material for nutrient analyses in each tube, the number of replicates of N and P tissue concentration was not sufficient for a complete ANOVA. Therefore, we tested each specific effect of treatment or treatment interaction separately.

To test the effects of fertilisation, liming and nematicide treatment on the vegetation composition, a Multivariate Analysis of Variance (MANOVA), which is more powerful than a univariate analysis (see Stevens, 1996), was performed.

MANOVA was also used to test the effects of fertilisation, liming and plant species on the composition of the plant- and fungal-feeding nematode community. The treatment effects on the individual nematode taxa were analysed using ANOVA. Prior to the analyses, the nematode numbers were ln(x+1) transformed.

Results

FIELD MANIPULATION EXPERIMENT

Both liming and fertilisation affected the soil pH. Liming, however, did not effectively increase the soil pH to a desirable level in all fields. The pH in the most productive fields O (pH 4.5) and B (pH 3.7) increased to 5.0 and 4.2, respectively, whereas the pH in the least productive fields C (pH 3.6) and K (pH 3.5) slightly increased to 3.7 and 3.6, respectively. The small effects of liming, particularly in the least productive fields, were probably caused

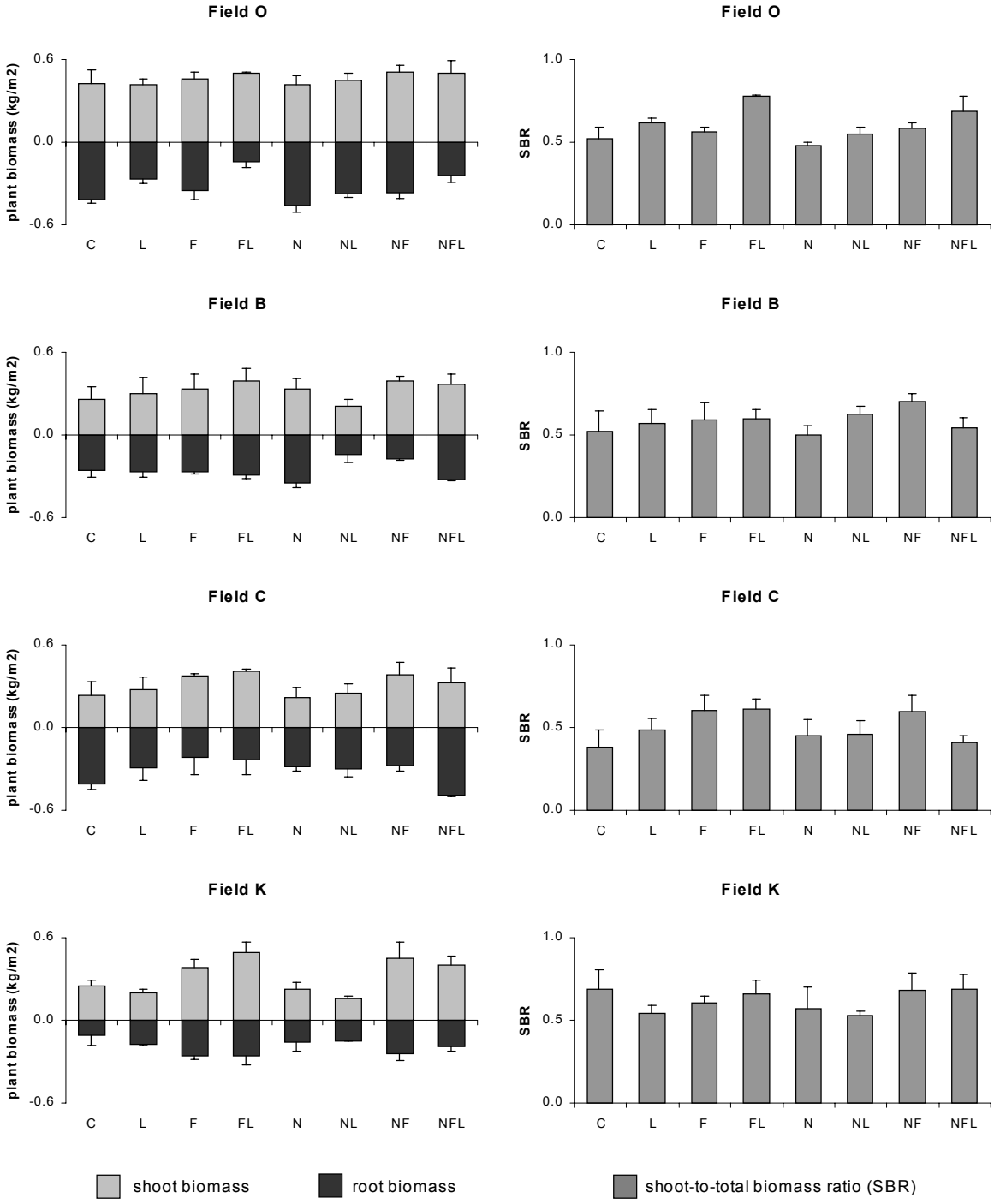


Figure 7.1. Mean (+s.e) shoot and root biomass, and shoot-to-total biomass ratio (SBR) of the vegetation in experimental quadrates of four grasslands (fields O, B, C, K) ranging from high to low producitivity, respectively. The quadrates were treated by different combinations of lime (L), fertilisers (F) and nematicides (N), or remained untreated (C).

by a low infiltration of the lime into the soil (see discussion). Fertilisation resulted in a slight, but statistically significant decrease of soil pH in all fields with 0.1.

Table 7.1. Results of an Analysis of Variance (ANOVA) testing the differences in the root, shoot and total biomass, and the shoot-to-total biomass ratio (SBR) of the vegetation in experimental plots of different sites (O, B, C, and K) after treatment with nematicides (+/-), fertilisation (+/-) and liming (+/-). ANOVA was used to test differences within each field and between fields.

	df	*F* values			
		Root biomass	Shoot biomass	Total biomass	SBR
Between sites					
Site (S)	3	2.87	4.00	4.46	1.85
S×Nematicide (N)	3	1.85	0.41	0.86	1.04
S×Fertilisation (F)	3	3.04	5.23 *	16.64 **	0.26
S×N×F	3	1.66	0.13	0.79	1.14
Within Field O					
N	1	1.97	0.51	2.06	1.48
F	1	75.86 *	30.10 *	131.07 **	23.30 *
N×F	1	0.03	0.21	0.01	0.05
Within Field B					
N	1	1.17	0.11	2.51	0.44
F	1	0.01	17.04	2.81	0.47
N×F	1	0.01	0.48	0.01	0.37
Within Field C					
N	1	2.34	0.28	0.09	8.40
F	1	0.17	13.73	151.30 **	3.66
N×F	1	53.64 *	0.03	4.77	12.84
Within Field K					
N	1	0.26	0.38	0.66	0.01
F	1	10.25	27.92 *	281.69 **	1.20
N×F	1	0.39	0.16	0.07	1.50

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

The plant biomass and shoot-to-total-biomass ratio (SBR) in all treatments are given in Fig 7.1. Since liming did not effectively increase the soil pH in all fields, we decided to focus on the effects of nematicide and fertilisation only. The statistical results are given in Table 7.1. In the following paragraphs we will describe the significant treatment effects on the basis of the ANOVA results of Table 7.1. References to the significant effects in Table 7.1 are given between brackets.

The application of nematicide had minor effects on the plant biomass in the field plots, although it reduced the plant-feeding nematode numbers by 68-85%. A significant effect of nematicide was found only in fertilised plots of field C, in which the root biomass increased with +72.2% (field C: N×F). In the unfertilised quadrates, however, nematicide treatment resulted in a -16.1% decrease of the root biomass.

Fertilisation (F) significantly affected plant biomass, but the average response of root, shoot and total biomass to fertilisation depended on the productivity of the fields. The root biomass significantly decreased (-7.5%) in the high-production field O after fertilisation, was unaffected in moderate-production fields B (+2.9%) and C (-4.5%), and tended to increase in low-production field K (+62.6%). The average shoot biomass increased in fields O, B, C, and K (+14.8, +35,3, +51.3 and +106.9%, respectively). Subsequently, the total biomass decreased in field O (-5.1%) and increased in fields B, C, and K (19.7, 19.8, and 88.7%, respectively). The SBR tended to increase after fertilisation in all fields (+21.1, +10.1, +24.8, and +13.4% in fields O, B, C, K, respectively), but the increase was significant only in field O.

The composition of the vegetation in the different treatments at the final harvest in 1998 is given in Fig 7.2. Sixteen months after the first treatment no statistically significant changes in the species composition of the vegetation in field O and field C were found. In field B only a positive effect of fertilisation on the proportions of *Anthriscus sylvestris* ($P = 0.011$) and the combined group of rare plant species (others; $P = 0.011$) was found. The major changes in vegetation composition were found in field K. in field K, fertilisation resulted in a strong reduction of the moss coverage ($P << 0.001$) and an increase of *Agrostis capillaris* ($P = 0.004$).

The numbers of plant- and fungal-feeding nematodes were strongly reduced by the application of nematicide in all fields at the end of 1997, eight months after the first treatments (Figure 7.3; Table 7.2). Fertilisation did not affect the nematode numbers and community composition in fields O and B. In field C, however, the plant- and fungal-feeding nematode density increased. Particularly, the numbers of *Pratylenchus* and *Hemicycliophora* significantly increased, while also *Paratylenchus* and Aphelenchidae, although not significantly, tended to increase after fertilisation. In field K the numbers of plant- and fungal-feeding nematodes tended to increase after fertilisation, but only a significant increase was found for Aphelenchidae in the treatments without nematicide application.

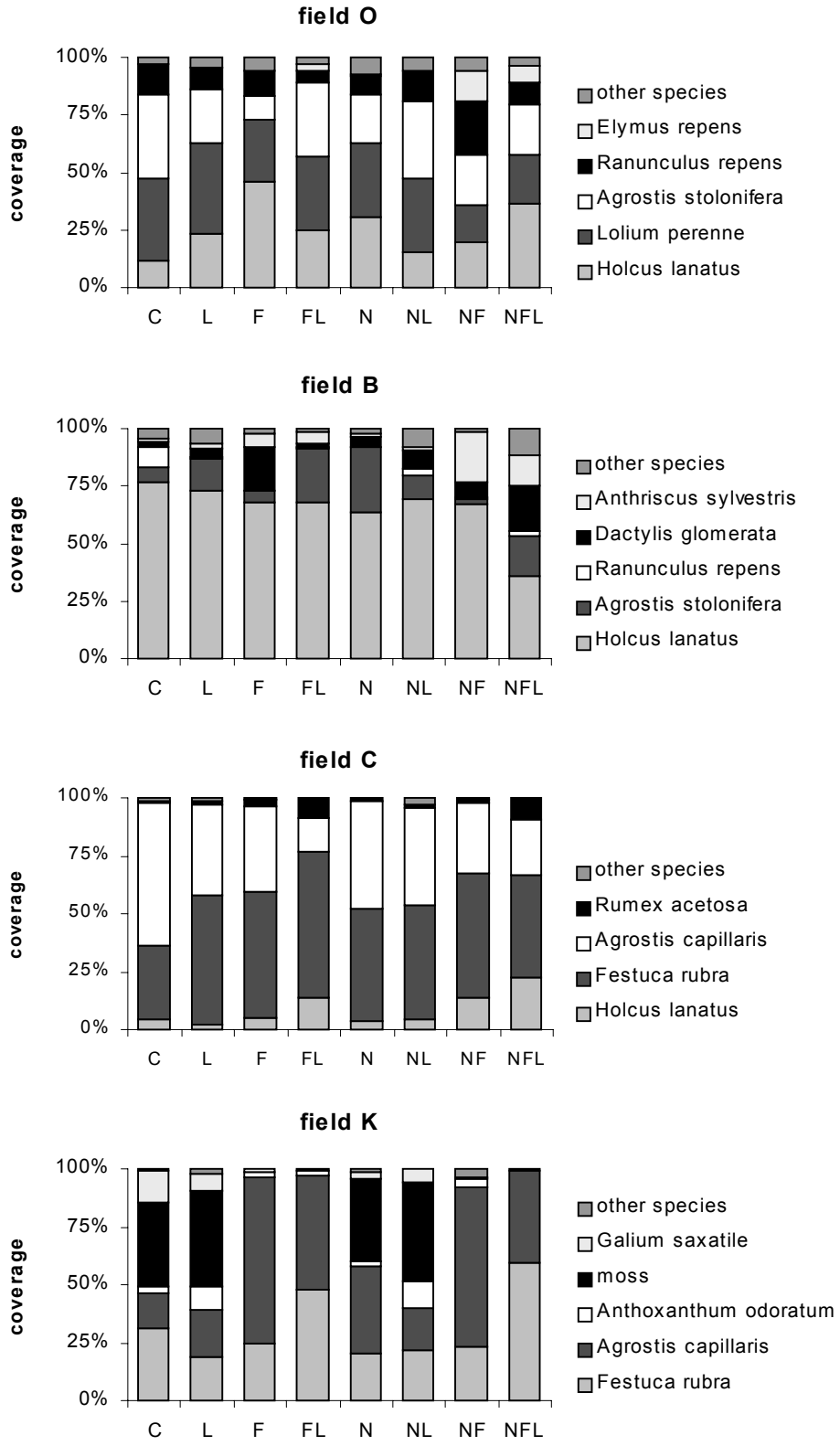


Figure 7.2. Mean relative cover of dominant plant species in experimental quadrates of fields O, B, C, and K that were treated by different combinations of lime (L), fertilisers (F) and nematicides (N), or remained untreated (C).

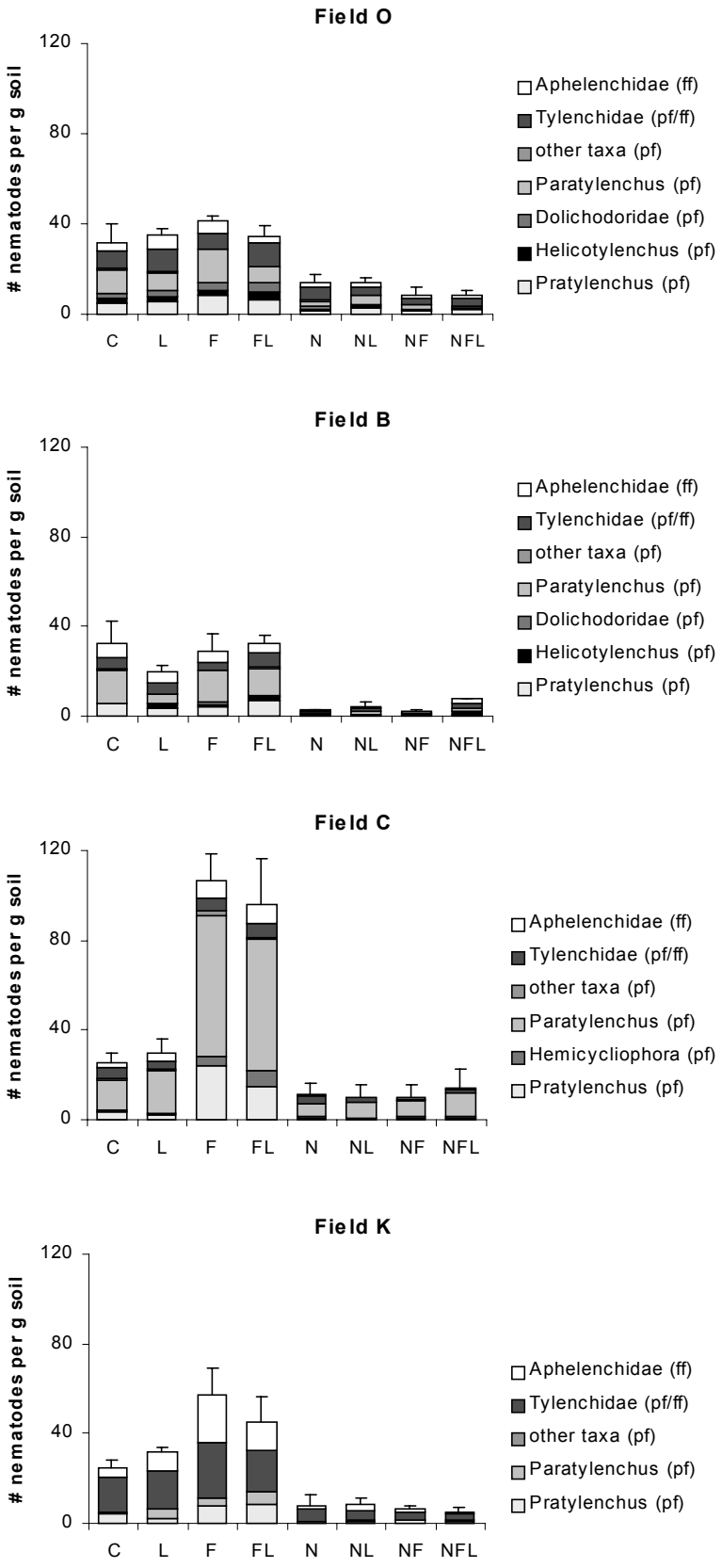


Figure 7.3. Mean numbers (+s.e) of plant-feeding (pf) and fungal-feeding (ff) nematodes in experimental quadrates of fields O, B, C, and K at different treatments. See Fig 7.1 for abbreviations.

Table 7.2. Results of an (Multivariate) Analysis of Variance ((M)ANOVA) testing the differences in the composition of the plant- and fungal-feeding nematode communities in the fields O, B, C and K under different treatment conditions. Treatments consisted of nematicide (N) and fertiliser (F) application.

	F values		
	N	F	N×F
Field O			
Pratylenchus (pf)	19.82***	0.08	0.77
Helicotylenchus (pf)	16.30***	0.04	0.88
Dolichodoridae (pf)	19.22***	0.09	2.05
Paratylenchus (pf)	21.50***	0.77	4.29
Others (pf)	1.09	0.24	0.58
Tylenchidae (pf/ff)	17.71***	0.30	0.10
Aphelenchidae (ff)	12.59**	1.20	0.14
Total community (pf+ff)	14.73***	0.31	2.23
Field B			
Pratylenchus (pf)	119.60***	0.16	2.24
Helicotylenchus (pf)	4.04	0.24	0.42
Dolichodoridae (pf)	2.14	2.59	2.95
Paratylenchus (pf)	33.88***	1.80	0.04
Others (pf)	7.15	0.09	0.00
Tylenchidae (pf/ff)	31.13***	0.03	0.00
Aphelenchidae (ff)	54.36***	0.01	0.44
Total community (pf+ff)	24.79***	0.85	1.28
Field C			
Pratylenchus (pf)	87.28***	21.60***	24.99***
Hemicycliophora (pf)	5.30*	5.68*	2.18
Paratylenchus (pf)	16.74***	4.26	2.48
Others (pf)	9.03**	0.34	0.34
Tylenchidae (pf/ff)	20.45***	0.60	5.50*
Aphelenchidae (ff)	29.78***	2.26	1.49
Total community (pf+ff)	20.64***	5.01*	4.21*
Field K			
Pratylenchus (pf)	3.25	0.96	0.12
Paratylenchus (pf)	11.29**	0.37	1.75
Others (pf)	0.74	0.00	1.93
Tylenchidae (pf/ff)	59.95***	0.04	2.24
Aphelenchidae (ff)	71.46***	0.89	14.40**
Total community (pf+ff)	19.79***	0.44	3.35*

$P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

pf = plant feeder; ff = fungal feeder; pf/ff = fungal/root hair feeder

TUBE PLANTING EXPERIMENT

The average plant biomass and SBR of plants grown in tubes are presented in Figure 7.4. In the following paragraphs we will describe the significant treatment effects on these plant parameters on the basis of the ANOVA results of Table 7.3.

Nematicide treatment effectively reduced the numbers of plant- and fungal-feeding nematodes (95-100% reduction; Figure 7.5). The effects on plant biomass, however, were relatively small. Nematicide treatment only significantly increased the root biomass of *F. rubra*. This biomass increase, however, was higher in field K (+67.3%) than in field O (+12.5%) (Table 7.3: N×S). The biomass of all other plant species was not significantly affected by nematicide treatment.

The supply of lime into the soil of the tubes resulted in a strong increase of the soil pH from 4.7 to 6.2 in soil of field O and from 3.8 to 5.8 in soil of field K. Liming as well as fertilisation significantly affected plant biomass and SBR of the plant species. These effects, however, were generally different between the sites and plant species (Table 7.3: (P)×F×S and (P)×L×S).

Lolium perenne, which is a characteristic species of the high-production field O, produced less root biomass (-45.8%), more shoot biomass (+75.1%) and a higher SBR (+92.4%) in field O than in the low-production field K. Fertilisation positively affected root biomass, shoot biomass and SBR of *L. perenne* in field O (+17.7%, +147.6%, +36.7%, respectively), in contrast to negative effects of fertilisation in field K (-37.4%, -50.6%, -22.0%, respectively). On the other hand, liming resulted in a decrease of root and shoot biomass of *L. perenne* in field O (-43.0% and –56.7%), and an increase in field K (+166.7% and +123.8%).

Holcus lanatus which is also a characteristic species of field O, was similarly affected by site, fertilisation and liming as *L. perenne*. *H. lanatus* produced less root biomass (-32.2%), more shoot biomass (+333.0%) and a higher SBR (+150.7%) in field O than in field K. The plant biomass of *H. lanatus* was not significantly affected by fertilisation, although the SBR slightly increased (+16.2%). Liming, however, resulted in a decrease of root biomass of *H. lanatus* in field O (-33.5%), and an increase of root and shoot biomass in field K (+57.0% and +71.7%).

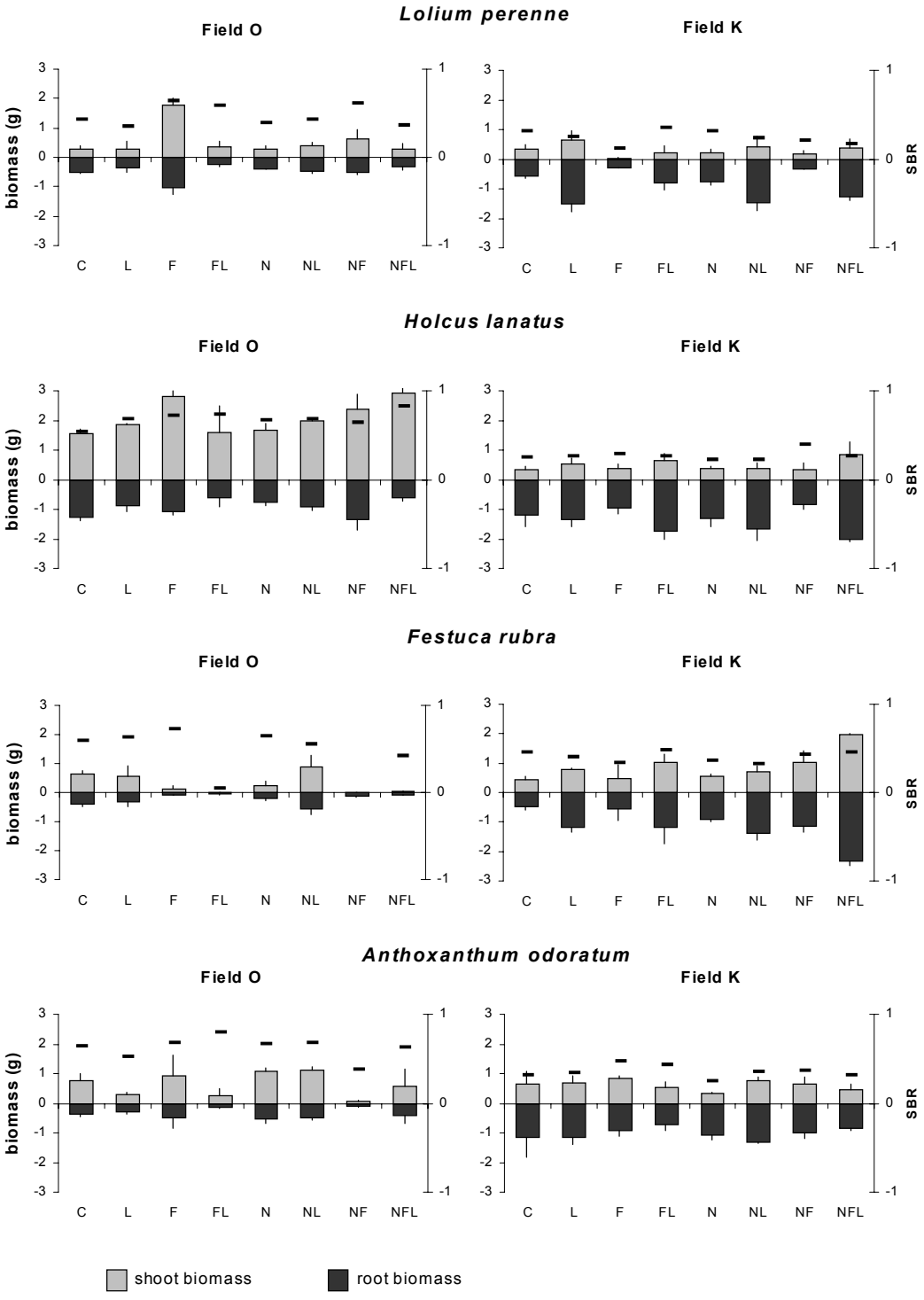


Figure 7.4. Mean (+s.e) shoot and root biomass of *Lolium perenne*, *Holcus lanatus*, *Festuca rubra* and *Anthoxanthum odoratum* in plastic tubes that were placed in fields O and K. The Mean shoot-to-total biomass ratio (SBR) is presented here by bold dashes. See Fig 7.1 for abbreviations.

Table 7.3. Results of an Analysis of Variance (ANOVA) testing the differences in the root and shoot biomass, and the shoot-to-total biomass ratio (SBR) between and within different plant species (*L. perenne*, *H. lanatus*, *F. rubra* and *A. odoratum*). Plants were grown in tubes, that were planted in two sites (fields O and K) under different treatment conditions. Treatments consisted of combinations of nematicide (+/-), fertiliser (+/-), and lime (+/-).

	F values				
	Between species	*Lolium perenne*	*Holcus lanatus*	*Festuca rubra*	*Anthoxanthum odoratum*
Root biomass					
Plant species (P)	24.22 ***				
(P)×Nematicide (N)	1.28	0.23	0.13	7.54 **	0.61
(P)×Fertilisation (F)	0.66	5.31 *	0.14	1.61	4.05
(P)×Liming (L)	2.81 *	9.28 **	0.28	11.90 **	0.05
(P)×Site (S)	8.07 ***	28.22 ***	12.12 **	92.56 ***	38.65 ***
(P)×N×F	0.89	0.10	0.17	1.77	0.56
(P)×N×L	0.15	3.07	0.91	0.31	1.54
(P)×N×S	0.96	3.08	0.43	6.05 *	0.03
(P)×F×L	0.41	2.53	0.07	0.01	0.33
(P)×F×S	4.52 **	8.90 **	0.12	8.66 **	0.01
(P)×L×S	4.93 **	51.80 ***	15.83 ***	8.54 **	0.03
(P)×N×F×L	0.54	2.32	0.13	0.00	0.62
(P)×N×F×S	1.08	1.65	1.25	0.87	0.51
(P)×N×L×S	0.19	1.97	0.01	0.73	0.67
(P)×F×L×S	2.52	4.23 *	6.27 *	0.89	0.99
(P)×N×F×L×S	1.02	0.35	0.93	1.78	0.67
df effect, df error	3, 128	1, 32	1, 32	1, 32	1, 32
Shoot biomass					
Plant species (P)	23.18 ***				
(P)×Nematicide (N)	0.69	0.80	0.51	1.13	0.02
(P)×Fertilisation (F)	2.26	0.31	2.50	1.28	2.58
(P)×Liming (L)	1.72	0.29	0.70	5.82 *	0.24
(P)×Site (S)	34.90 ***	3.19	89.17 ***	26.28 ***	0.22
(P)×N×F	1.93	0.27	0.50	2.06	2.84
(P)×N×L	0.29	0.76	1.33	0.81	3.39
(P)×N×S	1.58	0.46	0.80	3.13	0.78
(P)×F×L	0.68	3.14	0.15	0.04	0.38
(P)×F×S	7.59 ***	6.10 *	0.32	17.02 ***	3.31
(P)×L×S	0.57	5.71 *	1.39	2.70	0.30
(P)×N×F×L	0.62	0.58	2.57	0.08	0.06
(P)×N×F×S	1.03	3.79	0.14	1.56	2.02
(P)×N×L×S	0.00	1.19	1.27	1.54	1.06
(P)×F×L×S	2.18	2.87	2.65	2.16	1.64
(P)×N×F×L×S	0.63	0.32	0.45	0.98	0.52
df effect, df error	3, 128	1, 32	1, 32	1, 32	1, 32

Table 7.3 continued

	F values				
	Between species	*Lolium perenne*	*Holcus lanatus*	*Festuca rubra*	*Anthoxanthum odoratum*
SBR					
Plant species (P)	8.67 ***				
(P)×Nematicide (N)	1.06	0.60	0.54	0.09	3.31
(P)×Fertilisation (F)	2.78 *	0.82	5.38 *	3.75	0.88
(P)×Liming (L)	2.21	0.51	1.14	6.16 *	1.22
(P)×Site (S)	10.82 ***	16.88 ***	149.67 ***	9.03 **	45.91 ***
(P)×N×F	2.26	0.66	0.04	1.04	7.78 **
(P)×N×L	1.13	0.66	0.05	2.00	0.68
(P)×N×S	0.13	0.10	0.22	0.12	0.02
(P)×F×L	0.66	0.01	0.05	2.00	0.92
(P)×F×S	4.82 **	3.72	0.39	8.87 **	0.97
(P)×L×S	3.66 *	0.78	4.60 *	8.31 **	0.71
(P)×N×F×L	2.42	1.61	0.92	3.59	0.18
(P)×N×F×S	0.24	0.26	0.92	0.25	2.93
(P)×N×L×S	0.47	0.17	0.34	3.98	0.35
(P)×F×L×S	4.70 **	1.84	0.69	10.13 **	5.01 *
(P)×N×F×L×S	[1]	0.02	2.41	[1]	0.03
df effect, df error	3, 100	1, 24	1, 29	1, 22	1, 25

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.05$.
1) not enough replicates for statistical analyses

Table 7.4. Mean total nitrogen and phosphorus concentrations in the roots of four plant species grown in tubes that were planted in experimental plots of fields O and K under different treatment conditions. Treatments consisted of combinations of nematicide (+/-), fertiliser (+/-), and lime (+/-). The number of analysed samples is given between brackets.

	-nematicide				+nematicide			
	-fertiliser		+fertiliser		-fertiliser		+fertiliser	
	-lime	+lime	-lime	+lime	-lime	+lime	-lime	+lime
				Lolium perenne				
Field O								
N - root (mg/g)	12.5 (2)	13.4 (2)	14.4 (3)	16.7 (2)	13.0 (3)	13.1 (3)	16.9 (2)	15.3 (2)
P - root (mg/g)	1.82 (2)	1.57 (2)	2.14 (3)	2.22 (2)	1.68 (3)	1.67 (3)	2.01 (2)	2.16 (2)
Field K								
N - root (mg/g)	11.4 (3)	9.6 (3)	13.5 (3)	11.7 (3)	10.9 (3)	9.1 (3)	14.4 (3)	11.6 (3)
P - root (mg/g)	1.21 (3)	1.20 (3)	1.53 (3)	1.54 (3)	1.18 (3)	1.03 (3)	1.71 (3)	1.56 (3)
				Holcus lanatus				
Field O								
N - root (mg/g)	11.4 (2)	12.8 (2)	12.3 (3)	12.4 (2)	11.6 (3)	11.1 (2)	13.1 (3)	14.1 (3)
P - root (mg/g)	1.74 (2)	1.69 (2)	1.61 (3)	1.51 (2)	1.66 (3)	1.57 (2)	1.85 (3)	1.90 (3)
Field K								
N - root (mg/g)	9.9 (3)	9.0 (3)	11.5 (3)	10.7 (3)	10.4 (3)	9.0 (3)	10.9 (3)	11.4 (3)
P - root (mg/g)	1.07 (3)	1.07 (3)	1.35 (3)	1.34 (3)	1.15 (3)	1.04 (3)	1.29 (3)	1.38 (3)
				Festuca rubra				
Field O								
N - root (mg/g)	13.3 (2)	14.8 (2)	(0)	(0)	14.1 (1)	13.3 (2)	12.4 (1)	(0)
P - root (mg/g)	1.72 (2)	1.85 (2)	(0)	(0)	1.79 (1)	1.96 (2)	1.55 (1)	(0)
Field K								
N - root (mg/g)	12.1 (3)	11.3 (3)	13.1 (3)	11.2 (3)	11.8 (3)	10.0 (3)	14.8 (3)	11.8 (3)
P - root (mg/g)	1.26 (3)	1.51 (3)	1.36 (3)	2.01 (3)	1.33 (3)	1.46 (3)	1.93 (3)	1.99 (3)
				Anthoxanthum odoratum				
Field O								
N - root (mg/g)	14.7 (3)	13.3 (2)	13.0 (2)	16.5 (1)	13.6 (3)	13.9 (3)	15.3 (1)	11.0 (1)
P - root (mg/g)	2.15 (3)	1.99 (2)	2.37 (2)	2.74 (1)	2.25 (3)	2.07 (3)	2.34 (1)	2.12 (1)
Field K								
N - root (mg/g)	10.6 (3)	9.1 (3)	14.5 (3)	11.2 (3)	11.0 (3)	9.3 (3)	11.8 (3)	10.8 (3)
P - root (mg/g)	1.34 (3)	1.23 (3)	1.93 (3)	1.94 (3)	1.17 (3)	1.43 (3)	1.38 (3)	1.77 (3)

Table 7.5. Results of an Analysis of Variance (ANOVA) testing the differences in the root N and P concentration between and within different plant species (*L. perenne*, *H. lanatus*, *F. rubra* and *A. odoratum*). Plants were grown in tubes, that were planted in two sites (Fields O and K) under different treatment conditions. Treatments consisted of combinations of nematicide (+/-), fertiliser (+/-), and lime (+/-).

	F values				
	Between species	*Lolium perenne*	*Holcus lanatus*	*Festuca rubra*	*Anthoxanthum odoratum*
Root N concentration					
Plant species (P)	11.32 ***				
(P)×Nematicide (N)	0.79	0.01	0.33	0.00	2.16
(P)×Fertilisation (F)	6.62 ***	39.27 ***	25.37 ***	0.01	5.65 *
(P)×Liming (L)	1.27	5.04 *	0.27	3.34	7.33 *
(P)×Site (S)	3.21 *	47.22 ***	49.66 ***	6.17 *	37.68 ***
(P)×N×F	2.01	0.60	2.03	1.33	2.83
(P)×N×L	0.37	2.18	0.02	0.99	0.98
(P)×N×S	0.18	0.37	0.00	0.05	0.06
(P)×F×L	0.49	0.02	1.29	0.49	0.02
(P)×F×S	1.94	0.15	1.00	8.49 **	5.62 *
(P)×L×S	0.47	9.96 **	4.54 *	4.82 *	2.73
(P)×N×F×L	2.31	1.10	3.62	0.45	2.58
(P)×N×F×S	1)	0.10	3.08	1)	0.05
(P)×N×L×S	0.89	0.68	0.65	0.00	4.09
(P)×F×L×S	1)	0.00	1.23	1)	0.00
(P)×N×F×L×S	1)	0.27	0.05	1)	7.98 **
df effect, df error	3, 98	1, 27	1, 28	1, 19	1, 24
Root P concentration					
Plant species (P)	29.66 ***				
(P)×Nematicide (N)	2.07	0.39	1.19	1.88	2.20
(P)×Fertilisation (F)	7.11 ***	62.78 ***	15.94 ***	4.53 *	20.32 ***
(P)×Liming (L)	3.51 *	0.78	0.50	11.53 **	0.45
(P)×Site (S)	14.69 ***	91.35 ***	124.51 ***	5.78 *	73.84 ***
(P)×N×F	3.10 *	0.70	3.18	0.33	4.36 *
(P)×N×L	0.90	0.01	0.04	1.82	0.61
(P)×N×S	0.69	0.01	0.84	1.14	0.40
(P)×F×L	0.27	1.34	0.97	0.01	0.82
(P)×F×S	3.50 *	1.23	9.51 **	31.46 ***	3.27
(P)×L×S	1.21	0.27	0.10	3.05	1.79
(P)×N×F×L	0.80	0.07	1.40	0.60	0.61
(P)×N×F×S	1)	1.72	5.81 *	1)	0.01
(P)×N×L×S	3.50 *	2.03	0.07	3.49	4.16
(P)×F×L×S	1)	0.50	0.28	1)	0.09
(P)×N×F×L×S	1)	0.36	0.11	1)	0.28
df effect, df error	3, 98	1, 27	1, 28	1, 19	1, 24

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.05$.

1) not enough replicates for statistical analyses

On the other hand *F. rubra*, a characteristic species of the low-production field K, produced more root and shoot biomass and a lower SBR in field K than in field O (+415.9%, +190.3%, -21.1%, respectively). Fertilisation increased root biomass, shoot biomass and SBR of *F. rubra* in field K (+30.2%, +81.0%, +10.1%, respectively), while it decreased after fertilisation in field O (-80.8%, -93.7%, -34.1%, respectively). Liming increased the root and shoot biomass of *F. rubra* in both sites, but relatively more in field K (+98.5% and +83.1%) than in field O (+27.3% and +48.9%).

Anthoxanthum odoratum, another characteristic species of the low-production field K, also produced significantly more root biomass (+186.5%) and a lower SBR (-42.7%) in field K compared to field O. The shoot biomass of *A. odoratum*, however, was unaffected by site. Fertilisation and liming had no statistically significant effects on the growth of *A. odoratum*.

Interactive effects of fertilisation, liming and site were also found (Table 7.3: (P)×F×L×S). However, these results were not relevant in the present study and, therefore, not discussed.

The average N and P concentrations in the roots of the four plant species are presented in Table 7.4. In the following paragraphs we will describe the significant treatment effects on the N and P concentrations on the basis of the ANOVA results of Table 7.5.

Nematicide treatment did not have any main effects on root nutrient concentrations. Only after fertilisation, nematicide application negatively affected the root P concentration of *A. odoratum* (-15.3%, whereas no effect was found for the other plant species (Table 7.5: (P)×N×F).

Site, fertilisation and liming, however, significantly affected the root nutrient concentrations in the plant. The root N and P concentrations of all plant species were higher in the high-production field O than in the low-production field K (N: concentration: +25.0%, +19.4%, +13.3%, +26.2%, and P concentration: +39.3%, +39.5%, +10.4%, +47.9% for *L. perenne*, *H. lanatus*, *F. rubra* and *A. odoratum*, respectively). The site difference in root N and P concentration was smaller for *F. rubra* than for the other plant species (Table 7.5: P×S).

Fertilisation generally increased the root N and P concentrations relatively more in field K (N: concentration: +25.0%, +16.4%, +12.3%, +20.5%, and P concentration: +37.5%, +23.8%, +31.1%, +35.9% for *L. perenne*, *H. lanatus*, *F. rubra* and *A. odoratum*, respectively) than in field O (N: concentration: +21.7%, +10.8%, -11.0%, +0.6%, and P concentration:

+26.6%, +3.3%, +15.2%, +13.2% for *L. perenne*, *H. lanatus*, *F. rubra* and *A. odoratum*, respectively) (Table 7.5: P×F×S).

In field K, liming decreased the root N concentration of all plant species (-16.6%, -5.9%, -14.3%, -15.9% for *L. perenne*, *H. lanatus*, *F. rubra* and *A. odoratum*, respectively), whereas no significant lime effects on root N concentration were found in field O (+2.9%, +4.3%, +6.0%, -3.3% for *L. perenne*, *H. lanatus*, *F. rubra* and *A. odoratum*, respectively) (Table 7.5: P×L×S).

Higher order interactions between nematicide, fertilisation, liming and site on root N and P concentrations were also found. These results, however, were generally less reliable due to missing values in the data, or were irrelevant for the present study.

Table 7.6. Results of an (Multivariate) Analysis of Variance ((M)ANOVA) testing the differences in the composition of the plant- and fungal-feeding nematode communities in the rhizospheres of different plant species (P) in different treatments. Plant species were grown in tubes planted in the high-production field O and the low-production field K. Treatments consisted of combinations of fertiliser (F) and lime (L).

	F values						
	F	L	P	F×L	F×P	L×P	F×L×P
Field O							
Pratylenchus (pf)	3.16	4.44*	4.46**	3.56	1.96	1.41	2.40
Helicotylenchus (pf)	4.24*	0.18	9.56***	0.06	1.44	2.17	0.25
Dolichodoridae (pf)	16.36***	4.72*	1.22	1.76	1.80	0.57	0.41
Paratylenchus (pf)	0.30	20.41***	11.35***	2.42	2.51	4.17	0.16
others (pf)	5.13*	6.12*	0.50	2.20	1.12	1.46	1.55
Tylenchidae (pf/ff)	17.83***	1.16	7.74***	0.32	5.05**	0.33	1.19
Aphelenchidae (ff)	3.08	2.16	3.12*	0.69	0.25	0.44	0.58
Plant-feeders (pf)	5.62***	7.88***	2.92***	1.98	1.86	1.17	0.88
Fungal feeders (ff)	8.92***	1.32	4.71***	0.40	2.49*	0.30	1.06
Plant+Fungal feeders	5.03**	7.34***	2.98***	1.66	1.61	1.04	0.87
Field K							
Pratylenchus (pf)	0.49	35.24***	24.03***	0.42	0.45	2.04	1.42
Helicotylenchus (pf)	9.17**	0.33	11.62***	3.57	0.99	3.16*	3.35*
Paratylenchus (pf)	11.19**	0.07	27.62***	12.15**	1.83	10.29***	1.19
others (pf)	0.48	0.40	0.89	4.83*	0.89	0.56	0.65
Tylenchidae (pf/ff)	19.84***	15.07***	25.69***	19.01***	1.85	13.29***	0.76
Aphelenchidae (ff)	10.07**	42.93***	4.87**	1.22	0.58	0.69	0.84
Plant-feeders (pf)	8.19***	7.00***	11.83***	7.42***	1.26	3.50***	1.50
Fungal feeders (ff)	16.70***	25.12***	9.73***	10.64***	1.18	5.69***	0.84
Plant+Fungal feeders	6.95***	8.64***	10.04***	5.96***	1.15	3.17***	1.28

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

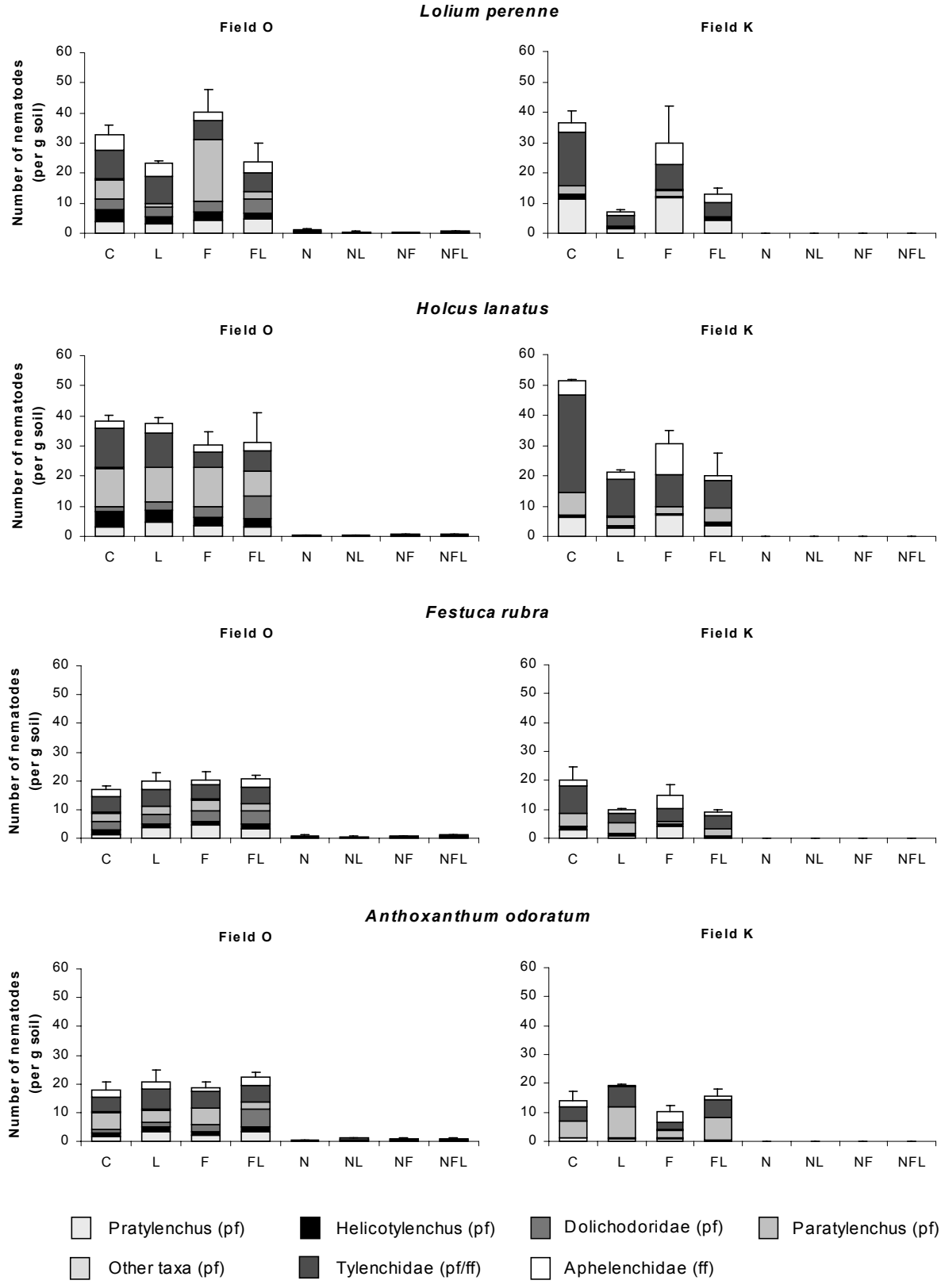


Figure 7.5. Mean numbers (+s.e) of plant-feeding (pf) and fungal-feeding (ff) nematodes under *Lolium perenne*, *Holcus lanatus*, *Festuca rubra* and *Anthoxanthum odoratum* in plastic tubes that were buried in fields O and K. See Fig 1 for abbreviations.

The composition of the plant- and fungal-feeding nematode community in the tubes was significantly affected by fertilisation, liming and plant species (Table 7.6 Figure 7.5).

On average the highest numbers of plant- and fungal-feeding nematodes, in both the high- and low-production field, were found under the plant species of high-production habitats, *L. perenne* and *H. lanatus* (Table 7.6: P).

Fertilisation on average reduced the numbers of *Helicotylenchus* and Tylenchidae in both fields. The numbers of Tylenchidae were particularly reduced after fertilisation of tubes with *L. perenne* and *H. lanatus* compared to tubes with *F. rubra* and *A. odoratum* (Table 7.6: F×P). Furthermore, the group of rare plant-feeding species (other taxa) in field O and *Paratylenchus* in field K was negatively affected by fertilisation. The numbers of Dolichodoridae in field O and Aphelenchidae in field K, however, increased when fertiliser was applied.

Liming had a slight positive effect on the numbers of *Pratylenchus* and Dolichodoridae in field O, but reduced the numbers of *Paratylenchus* and the group of rare plant-feeding species. The decreased number of *Paratylenchus* in field O after liming, however, was found only in tubes with *L. perenne* (Table 7.6: L×P). In field K, liming generally reduced the numbers of *Pratylenchus* and Aphelenchidae. The effect of liming on the average numbers of Tylenchidae, *Paratylenchus* and *Helicotylenchus* in field K depended on the plant species (Table 7.6: L×P). Their numbers always decreased under *L. perenne* and increased under *A. odoratum*, whereas intermediate effects were found under *H. lanatus* and *F. rubra*.

In field K also some interactive effects between fertilisation and liming on the numbers of nematodes were found (Table 7.6: F×L). The negative effect of liming on the numbers of Tylenchidae and *Paratylenchus* was found in the unfertilised treatments, whereas a slight positive effect of liming was found when fertilisers were applied. The opposite effect was found for the group of rare plant-feeding species.

Discussion

Effects of nematodes on vegetation

The succession in the studied hayfields of the Drentse A nature reserve is primarily driven by the reduced availability of nutrients in the soil in the years after the application of fertilisers

stopped (Bakker 1989; Olff and Bakker, 1991; Olff et al., 1994). The importance of the nutrient availability for plant succession was also demonstrated in the present field study in which fertilisation and liming considerably affected the productivity and composition of the plant community.

Root herbivores could affect the succession by affecting plant nutrition, colonisation and productivity (Brussaard et al., 1996). In line with Brussaard (1998) we expected that plant-feeding nematodes would be more damaging under nutrient-limited growth conditions. Fertilisation in field plots of the low-production field C, however, increased the nematode numbers to such a level, that nematicide treatments in these fertilised plots resulted in a significant increase of plant biomass (+72%). Thus in contrast to our hypothesis, fertilisation may also stimulate damage caused by nematodes, which reduced the benefit of fertilisation to plant productivity.

Furthermore, we found that in a low-production grassland, the dominant plant species of this site, *F. rubra*, tended to be more affected by nematode herbivory than plant species of high-production habitats, although no statistically significant difference was found (the mean root biomass of *F. rubra* increased with 67.3%, compared to 20.5%, 11.5% and 7.0% of *L. perenne*, *H. lanatus* and *A. odoratum*, respectively). The apparent sensitivity to nematode infestation of *F. rubra* possibly resulted from the presence of specific pathogen complexes in field K. In dune plant communities species-specific pathogen complexes were assumed to be responsible for the low vigour of plant species that were grown on soil taken from its own rhizosphere (Van der Putten et al., 1993). The high abundance of *F. rubra* in the vegetation of field K might have resulted in the build-up of pathogen complexes that are specific for this plant species. Such species-specific suppression of plant species, however, was not found for early-successional plant species in the high-production field O. Furthermore, species determinations of plant-feeding nematode communities under different plant species showed no obvious differences in the nematode species composition between plant species (this study; chapters 2, 5 and 6). These results seem to indicate that host-specificity of nematode communities is not a common property in the studied grasslands. On the other hand, plant-feeding nematodes may act as key species in pathogen complexes by creating entries in the roots which can stimulate the colonisation of roots by pathogenic micro-organisms (De Rooij-van der Goes, 1995; Van der Putten and Van der Stoel, 1998). In such a way even generalist nematodes can indirectly contribute to species-specific suppression of plant species.

Except for the fore-mentioned cases, the application of nematicide generally had small or no effects on the plant biomass in the experimental plots, although it effectively reduced the numbers of plant- and fungal-feeding nematodes. These minor effects of nematicide application on plant productivity were supported by earlier experiments in which the numbers of belowground herbivores in grasslands were manipulated by pesticides (Seastedt et al., 1987; Todd et al., 1992), but are in contrast to similar studies in other grasslands in which biomass increases were found of 25 to 59% in natural grasslands (Smolik, 1977; Stanton et al., 1981; Ingham et al., 1986b; Ingham and Detling, 1990) and even up to 172% in sown monocultures (Hoveland et al., 1975; Giblin-Davis et al., 1988).

Since the total number of plant- and fungal-feeding nematodes in our study sites was relatively high compared to other grassland ecosystems (De Goede and Bongers, 1998), we can only speculate why the exclusion of these nematodes did in general not result in a positive plant response. Recent studies have shown that herbivory might stimulate root exudation and subsequently enhance microbial activity in the soil (Bardgett et al., 1998; Bardgett et al., 1999b; Denton et al., 1999; Yeates et al., 1999a,b). As a result of that the nutrient availability was found to increase and even resulted in positive effects on plant growth at moderate levels of herbivory (Bardgett et al., 1999a). On the other hand, the application of nematicide also resulted in a strong reduction of non-target animals such as saprotrophic nematodes, which might stimulate plant growth by enhancing the nutrient turnover in the soil. Such positive effects of soil fauna on nutrient availability for plant root uptake can offset the negative effects of belowground herbivory (Seastedt et al., 1988).

Moreover, nematode herbivory might affect the species composition of the vegetation, by altering plant competition or inhibiting seedling colonisation, without affecting net primary production. Plant species could benefit from nematode infestation of neighbouring plants by a reduced competition for resources (Van der Putten and Peters, 1997; chapter 6). Particularly, when herbivory results in an increased nutrient availability in the soil the growth of neighbouring plants could be stimulated (Bardgett et al., 1999a,b). In permanent grasslands the impact of nematode herbivory on the colonisation and establishment of plant seedlings in the vegetation might be of particular importance. The nitrogen-rich seedlings are attractive food sources for young nematodes (Mattson, 1980) and are particularly vulnerable for infestation by nematodes or other pathogens (Marschner, 1995). The exclusion of nematodes might, therefore, increase seedling survival and subsequently affect the species composition of the plant community.

Nevertheless, we found no changes in the plant species composition after nematicide application. Presumably, the successional changes in the grasslands of the Drentse A nature reserve are relatively slow and resulted from gradual replacements of species on a small scale within the vegetation. Furthermore, the establishment of seedlings within a closed grassland vegetation might also be difficult in the absence of nematodes due to the high competition for resources, and in particular for light. We assume, therefore, that the experiment should be continued for some years before major shifts in the composition of the plant community can be observed. The short-term results, however suggest that plant-feeding nematodes do not have a dominant role in the vegetation succession.

Effects of vegetation on the nematode community

In the present study we found several indications that alterations in the nitrogen concentrations of plant tissues affected the number of plant- and fungal-feeding nematodes in the soil. Firstly, we found that fertilisation in the low-production fields increased both the root N concentration and the numbers of plant- and fungal-feeding nematodes. Secondly, liming in the low-production fields reduced root N concentration and this coincided with a decrease of the number of plant- and fungal-feeding nematodes under *L. perenne* and *H. lanatus*.

Nitrogen is an essential nutrient for herbivores and in particular for young developing animals (Mattson, 1980; White, 1984; Seastedt, 1985). In most plant tissues, however, the N availability for herbivores is limited. Reduced plant quality, for example, was assumed to be the main factor explaining the lower density and altered species composition of plant-feeding nematodes with time of non-fertilisation (chapter 2: Verschoor et al., 2001a). Management practices that increased the plant N concentration are considered to be beneficial for herbivore nematode populations (Sohlenius and Boström, 1986; Yeates, 1987; Todd, 1996).

Nematode densities could also have been altered by changes in root biomass. Generally, nematode numbers are positively correlated with plant productivity (Yeates, 1987). However, we found no indications that standing root biomass significantly affected nematode density. The highest nematode densities were found in fertilised quadrates of field C in which the root biomass was unaffected. Furthermore, liming increased plant biomass in field K, but resulted in a decrease of the nematode densities (compare the results in Fig 7.4 and Fig 7.5). This demonstrates that plant quality rather than plant quantity affected the nematode numbers.

Despite an assumed limited availability of N for herbivores, a positive relationship between plant N concentration and nematode density could not always be found. For example, fertilisation significantly affected nitrogen concentrations of plants grown in tubes, but increased nematode densities in the field plots only and not in the tubes. We assume that this absence of nematode increase in the fertilised tubes resulted from a high suppression of nematodes by fertilisation. It is well-known that high amount of inorganic nitrogen fertilisers can suppress the numbers of nematodes (Rodríguez-Kabana, 1986). Detrimental effects of fertilisers were also found in pot trials in the greenhouse (chapters 5 and 6) and were observed in the present study two weeks after the second application of fertilisers (unpublished data). In the field plots, the dense vegetation cover could have resulted in a higher plant nutrient uptake and a slower infiltration of nutrients into the soil. In the tubes, however, nematodes were more exposed to the nutrient solution. We assume, therefore, that a greater exposure of the tube soil to the applied nutrient solutions resulted in a relatively high reduction of the nematode population at the start of the experiment. Consequently, nematode densities in the fertilised tubes could have been relatively low in spite of a higher plant N concentration.

Furthermore, liming of the field plots had no effects on nematode densities, whereas it significantly affected the nematode densities and plant N concentrations in the tubes. In the field plots, however, the lime was added as powder on top of the vegetation and hardly infiltrated the soil of these plots, whereas in the tubes it was mixed throughout the soil with significant effects on pH. In fields C and K, liming did not affect the pH in the plots. Only in the high-production fields O and B the soil pH slightly increased, probably due to earthworm activity, which was almost absent in fields C and K. In contrast, in the tube experiment liming resulted in a significant increase of the soil pH, in significant plant responses, and subsequently in changes in the nematode densities.

Moreover, liming differentially affected the root N concentrations of the four plant species, but no positive relationship between N concentration and nematode numbers was found. Liming reduced root N concentration of *A. odoratum* which coincided with an increase of the numbers of nematodes. In contrast the root N concentration of *F. rubra* did not change by liming, but the numbers of nematodes decreased. The application of $CaCO_3$, however, not only affected plant N concentration, but also plant P concentration and probably the plant Ca concentration (not determined). Calcium has essential structural functions such as regulation of membrane permeability and strengthening of the cell walls and is of importance for determining the susceptibility of plant tissues to fungal infections (Marschner, 1995). We

assume that an increased root Ca concentration hampered the penetration of cell walls by plant-feeding nematodes, which might explain why particularly the migratory endoparasite *Pratylenchus* decreased after liming (this study; Dmowska and Ilieva, 1995).

The highest numbers of plant- and fungal-feeding nematodes were found under *L. perenne* and *H. lanatus*, that are species characteristic of high-production habitats. Plant species of high-production habitats are generally characterised by a higher nutrient uptake rate, a higher growth rate, a shorter tissue life span and a lower tissue density than plant species of low-production habitats (Lambers and Poorter, 1992; Ryser, 1996a,b). These characteristics contribute to a higher palatability of plant species of high-production habitats (Southwood et al., 1986; Coley, 1988). Such higher palatability may result from a higher nitrogen concentration (Mattson, 1980; Lambers and Poorter, 1992) or a lower physical-chemical defence (Edwards-Jones and Brown, 1993) in fast-growing plants. We found no relationship between the plant N concentrations of different plant species and the number of nematodes in their rhizosphere. However, nutrients can be located in plant tissues or cell structures that are inaccessible for sap-sucking nematodes. Total N, therefore, may not always be a good indicator of available N (Curry, 1994).

Conclusions

Plant species of high-production habitats were relatively more affected, in terms of biomass and nutrient concentration, by nutrient limitation or soil pH than species of low-production habitats. We found no support from the field for our hypothesis that under nutrient-poor conditions, early-successional plant species of high-production habitats will be more affected by nematode infestation than late-successional plant species of low-production habitats. The assumption that a high plant productivity at a high nutrient availability offsets damage caused by nematodes due to lower density of nematodes per gram root could not be justified. Fertilisation indeed stimulated plant productivity, but could also considerably increase the numbers of plant-feeding nematodes and subsequently nematode damage to the plant. We conclude, therefore, that an increased damage caused by nematodes can reduce the benefit of fertilisation to plant productivity. Under natural, unfertilised conditions, however, we found no evidence that plant-feeding nematodes significantly affected plant productivity in the

studied grasslands. Thus, the results of our study suggest that plant-feeding nematodes do not have a dominant role in reversed vegetation succession.

The plant- and fungal-feeding nematode community itself, however, was strongly affected by plant species, fertilisation and liming. We found indications that the numbers of nematodes were particularly affected by the concentration of nitrogen in the plant roots. Gradual changes in the soil and plant nutrient status might, therefore, strongly affect the density and species composition of the plant-feeding nematode community during vegetation succession.

VERSCHOOR, B.C., ILIEVA, Z., DE GOEDE, R.G.M. Host-specificity of plant-feeding nematode species and its relationship to grassland succession. Submitted to *Nematology*.

8. Host-specificity of plant-feeding nematode species and its relationship to grassland succession

Abstract Host specificity of soil-borne pathogens, including nematodes, is supposed to be an important factor affecting spatio-temporal variation in natural vegetation. Direct evidence for the presence of host specificity in plant-feeding nematode communities of grasslands is, however, poor. Therefore, we examined the host specificity of six plant-feeding nematode species for six plant species. Both the nematode and plant species were representatives of different stages of succession in former agricultural grasslands. In these grasslands fertiliser application was stopped while haymaking continued, resulting in a reduced nutrient availability in the soil and subsequent changes in the plant and nematode communities. It was hypothesised that host specificity exists for the different nematode species of the grasslands. Furthermore, we tested whether plant species with a life strategy adapted to high-production grasslands were better hosts for plant-feeding nematodes than plant species adapted to low-production grasslands due to a supposed higher palatability of the former.

For all nematode species host-specific differences in reproduction were found. No evidence was found that plant life strategy affected the reproduction rate of plant-feeding nematodes. Furthermore, we found no relationship between the reproduction rate of nematodes on different plant species and their abundance under the same plant species in the field. We concluded, therefore, that spatio-temporal distributions of plant-feeding nematodes do not mainly depend on the presence of host plant species, but must to a large extent depend on other environmental factors such as interspecific competition and abiotic conditions.

Introduction

Recently there is an increasing interest for the role of soil-borne pathogens in processes leading to spatio-temporal variation of natural vegetation (Van der Putten and Van der Stoel, 1998, Van der Putten, in press). Soil pathogen complexes, comprised of pathogenic fungi and plant-feeding nematodes, are thought to contribute to vegetation succession in coastal sand dunes (Van der Putten et al., 1993) and permanent grasslands (Olff et al., 2000; Verschoor et al., 2001a). Experimental studies showed that plants which were grown in their own soil were generally more inhibited in growth than plants which were grown in soil of a preceding plant species (Van der Putten et al., 1993). These results indicate the presence of specific pathogen complexes in the root zones of different plant species. The development of such host-specific pathogen complexes might result in a competitive replacement of a dominant plant species by its successor (Van der Putten and Peters, 1997). Host specificity of soil-borne pathogens such as pathogenic fungi and nematodes is, therefore, supposed to be an important factor in successional processes.

On the other hand Southwood et al. (1986) suggested a more general relationship between the palatability of plant species and their life expectancy. Fast-growing plant species of high-production habitats, which generally have a shorter life expectancy (Ryser, 1996a,b), therefore, may have a higher palatability to herbivores than slow-growing species of low-production habitats. The results of earlier experiments presented in this thesis (chapters 5, 6 and 7) were in agreement with this hypothesis, showing higher densities of plant-feeding nematodes under plant species characteristic of high-production grasslands than under those of low-production grasslands. It was concluded, therefore, that in addition to progressing nutrient stress, species-specific differences in tolerance to (generalist) plant-feeding nematodes, rather than host specificity, determine the plant species replacement during reversed succession in grasslands (chapter 6).

Information on host specificity of nematodes in grasslands and natural ecosystems, however, is still poor due to a lack of studies in such ecosystems (see reviews by Cook and York, 1980 and Cook and Yeates, 1993). Particularly, thorough information on population dynamics and host specificity of nematodes other than obligate endoparasites on grasses is lacking, even though they can have a detrimental effect on grassland productivity (Cook and York, 1980). In this study, we examined the level of host specificity of six plant-feeding nematode species that were dominant in high-production grassland sites and/or in low-

production grassland sites. These sites are part of a successional series of grasslands that differ in the time without fertiliser addition (chapter 2: Verschoor et al., 2001a). The nematode population growth on six different plant species, which were representatives of different successional stages, was examined in time. As it is assumed that fast-growing plant species adapted to high-production sites have a higher palatibility to herbivores than species of low-production sites (chapter 5), we will test whether the reproduction of nematodes is higher on plant species of high-production grasslands than on plant species of low-production grasslands. The relationship between nematode and host distribution in the field and its significance for vegetation succession is discussed.

Materials and Methods

Soil sampling and treatment

A loamy sand soil was collected in the upper 25-cm soil layer of a grassland site next to the Biological Station in Wijster, The Netherlands. The soil was sieved (1 cm mesh size) to remove roots and coarse material, thoroughly mixed, and finally sterilised by gamma-irradiation (20 kGy).

Collecting and growing of plants

Plant seeds of different grass species were collected in 1997 in grasslands of the Drentse A nature reserve, The Netherlands. The fields in this study area have been managed according to standard agricultural practice typical for permanent grassland until fertilisation was stopped, while haymaking continued to reduce the nutrient availability in the soil. Such management resulted in the replacement of fast-growing light-competing plant species by slow-growing nutrient-competitors (Bakker, 1989; Olff and Bakker, 1991). For the experiment six plant species were chosen which represented different stages of succession. *Lolium perenne* L., *Holcus lanatus* L. and *Agrostis stolonifera* L. were dominant species of the high-production early-successional stages, whereas *Festuca rubra* L., *Anthoxanthum odoratum* L. and *Agrostis capillaris* L. represented the low-production vegetation of the late-successional stages. Seeds of these plant species were germinated in Petri dishes filled with

moist sand in the greenhouse two weeks prior to planting. In February 1998 pots were filled with 200 g soil. Three seedlings of the same species were planted in each pot.

The pots were placed in a randomised arrangement in a climate chamber (photon flux density 120 μE m^{-2} s^{-1} (11,000 lux, day 16 h at 20 °C and night 8 h at 16 °C, relative air humidity 70%). The position of the pots was randomly changed once a week to avoid interference by possible local environmental differences. The pots were watered daily by spraying and once a week the pots were set to a soil moisture content of 20% (w/w) by weighing. To meet increasing plant requirements, 5 ml of a full-strength Hoagland solution was supplied per pot in week 5, 8, and then once a week from week 10 to 14. We used minimal fertiliser supplies for plant growth, to minimize the negative effects of fertilisation on the numbers of plant-feeding nematodes (personal observation).

NEMATODE COLLECTION AND TREATMENT

Six plant-feeding nematode species, *Aglenchus agricola* (de Man, 1884), *Paratylenchus veruculatus* (Wu, 1962), *Paratylenchus nanus* (Cobb, 1923), *Geocenamus nanus* (Allen, 1955), *Helicotylenchus pseudorobustus* (Steiner, 1914) and *Pratylenchus* (mixture of 70% *P. crenatus* (Loof, 1960) and 30% *P. fallax* (Seinhorst, 1968)) were examined for their host specificity. *P. nanus*, *G. nanus* and *H. pseudorobustus* were dominant species of the high-production grasslands, whereas *P. veruculatus* dominated the low-production grasslands. *A. agricola* and *Pratylenchus* spp were present at all grasslands (chapter 2). In our experiment monocultures of *A. agricola*, *P. nanus* and *G. nanus*, which were isolated from our study area and cultured in pots with mixtures of grass species, were used as inoculum. In case of *P. veruculatus*, *H. pseudorobustus* and *Pratylenchus* no monocultures were available. Therefore, these species were inoculated as species mixtures of nematodes extracted from soil of a low-production grassland and a high-production grassland, in which *P. veruculatus* and *H. pseudorobustus* were the dominant plant-feeding species, respectively, or extracted from root samples collected in the low-production grassland to collect *Pratylenchus*. Inoculum densities were set at approximately 100 specimens for *A. agricola*, *P. veruculatus, P. nanus*, *G. nanus,* and *H. pseudorobustus* and approximately 50 specimens for *Pratylenchus*. These nematodes were added to the pots two weeks after seedlings were planted. The development of the nematode populations was examined 7, 27, 46, 71 and 110 days after inoculation by harvesting three replicates per treatment. Since we focussed on nematode-plant interactions

that could actually occur in the field, we examined the following nematode-plant combinations in time: *A. agricola*, *P. nanus*, *G. nanus, H. pseudorobustus* and *Pratylenchus* in pots grown with *L. perenne*, *H. lanatus* and *A. stolonifera*, and *A. agricola*, *P. veruculatus*, and *Pratylenchus* in pots grown with *F. rubra*, *A. odoratum* and *A. capillaris*. All other nematode-plant combinations, however, were examined only at the first harvest after 7 days and final harvest after 110 days.

HARVEST, NEMATODE EXTRACTION AND COUNTING

The nematodes were extracted from the soil using a modified Oostenbrink elutriator (Oostenbrink, 1960) and incubated 48 hours on a double cottonwool filter (Hygia milac filter). To extract *Pratylenchus* from both the soil and roots, roots in the top sieve of the Oostenbrink elutriator were cut into pieces of approximately 1 cm and macerated for 5 minutes in a domestic blender. The root suspension was then added to the soil-nematode suspension on the cottonwool filter. After extraction 5% of the nematode suspension was counted under a low magnification inverted microscope and the proportions of adults and juveniles determined. The nematode numbers were corrected for nematode losses that occurred during the extraction procedure according to the equations 1 and 5 of Verschoor and De Goede (2000). Since the loss corrections are size dependent and nematode lengths were not measured in this experiment, we approximated juvenile length to be half the length of an adult nematode (Verschoor and De Goede, 2000). For all treatments, except the *Pratylenchus* treatment, plant roots were collected after nematode extraction, dried at 70 °C for 48 hrs and weighed.

DATA ANALYSES

The intrinsic rates of nematode reproduction (IRR) and root growth (IGR) were calculated for each time interval. Therefore, the increase in nematode density or root biomass ($\Delta N=N_2-N_1$) was divided by the interval time ($\Delta t=t_2-t_1$) and the total nematode density or root biomass at the start of each preceding time interval (N_1). Thus, $IRR_2=\Delta N/(N_1\times\Delta t)$.

To test for differences in the number of nematodes that developed in the presence of the different plant species an Analysis of Variance (ANOVA) was done. In order to meet the rules of normality and homogeneity the nematode data were ln(x+1) transformed. The effect

of plant species and harvest time on the proportion of juveniles of each nematode species was tested by ANOVA after the proportions were arcsin transformed.

Results

Since the root biomass of each plant species did not significantly differ between the nematode treatments at the final harvest, we have calculated the average root biomass for all nematode treatments together. The root biomass of the six plant species increased in time (Fig 8.1a), but the intrinsic growth rate strongly decreased after 6 weeks and almost ceased after day 70 for most plant species, except *F. rubra* (Fig 8.1b). Also the number of nematodes for each species, except *Pratylenchus* spp., increased in time, although significant differences in density, intrinsic growth rate, and proportion of juveniles were observed between the different plant species (Fig 8.2).

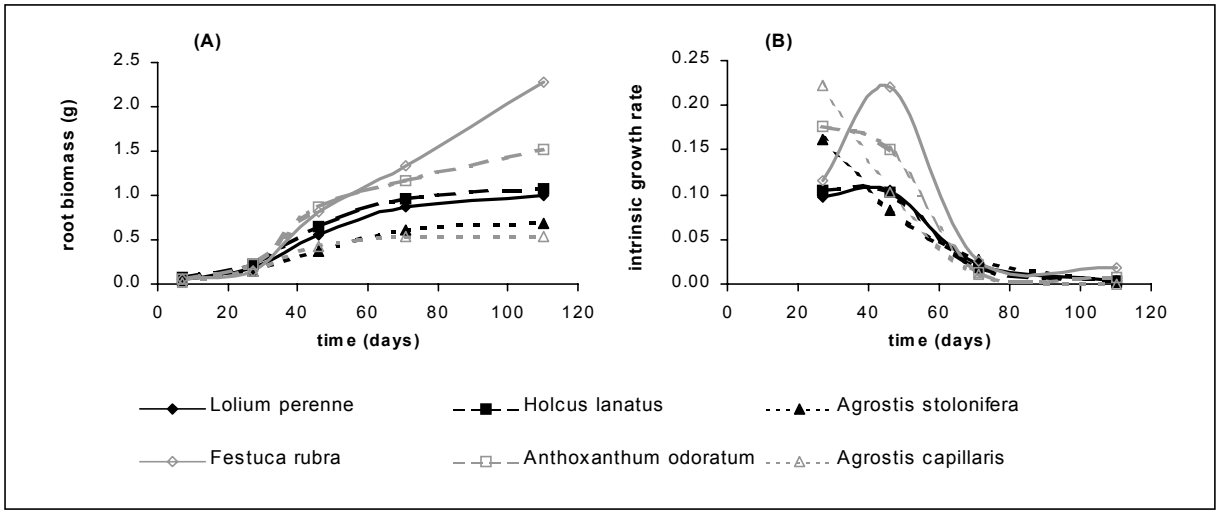


Figure 8.1. The total root biomass per pot (A) and intrinsic growth rate (B) of six plant species *L. perenne*, *H. lanatus*, *A. stolonifera*, *F. rubra*, *A. odoratum*, and *A. stolonifera*. Intrinsic growth rate is measured as the biomass increase per g for each time interval. The root biomass is calculated as the average biomass of plants that are grown at different nematode treatments.

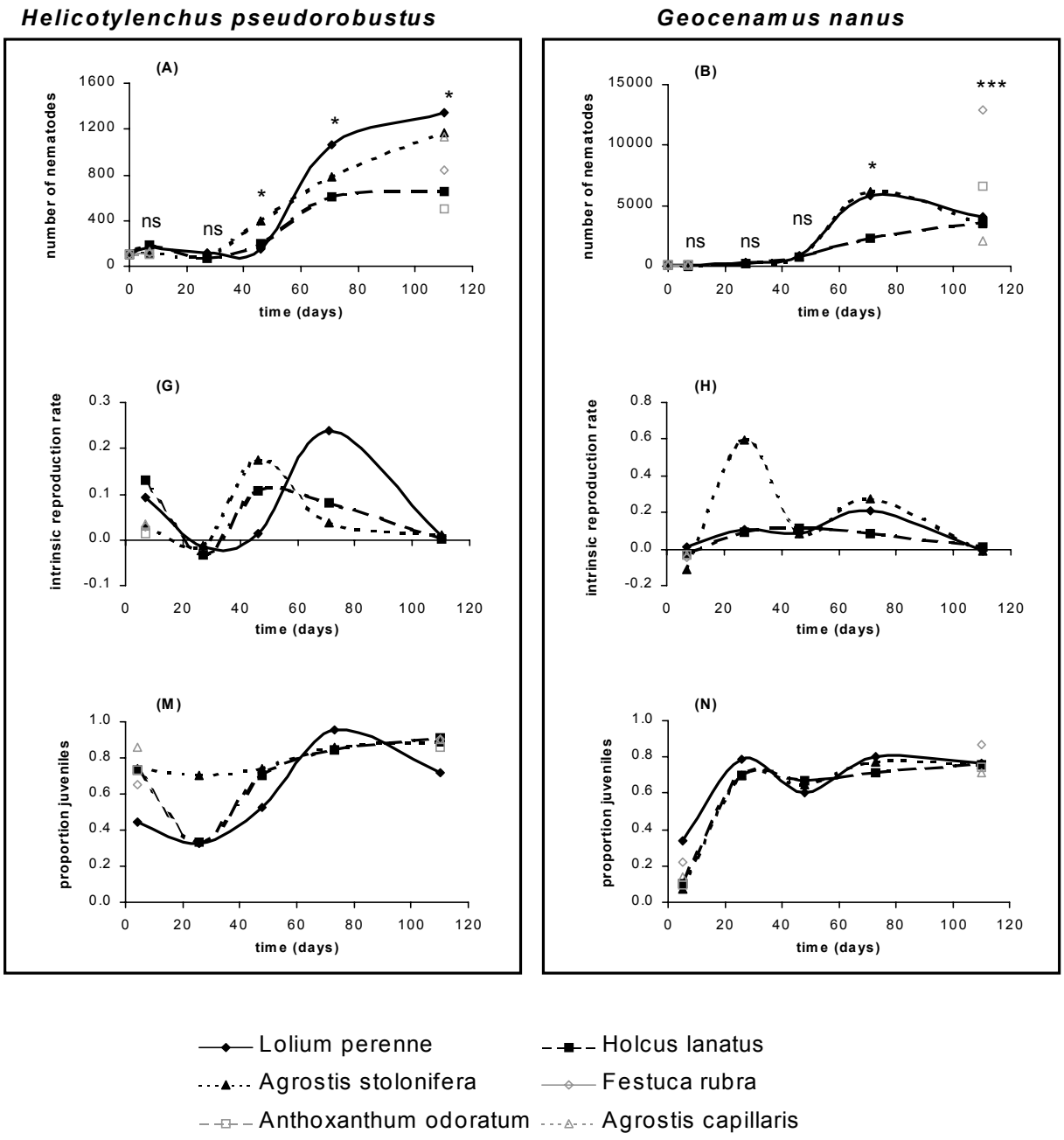


Figure 8.2. Population development (A-F), intrinsic reproduction rate (G-L), and proportion of juveniles (Fig M-Q) of six nematode species under the plant species *L. perenne*, *H. lanatus*, *A. stolonifera*, *F. rubra*, *A. odoratum*, and *A. stolonifera*. The nematode densities are presented as totals per pot. Intrinsic rate is measured as the population increase per nematode for each time interval. Significant differences (tested by ANOVA) between the treatments for each harvest are presented by: *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns = not significant.

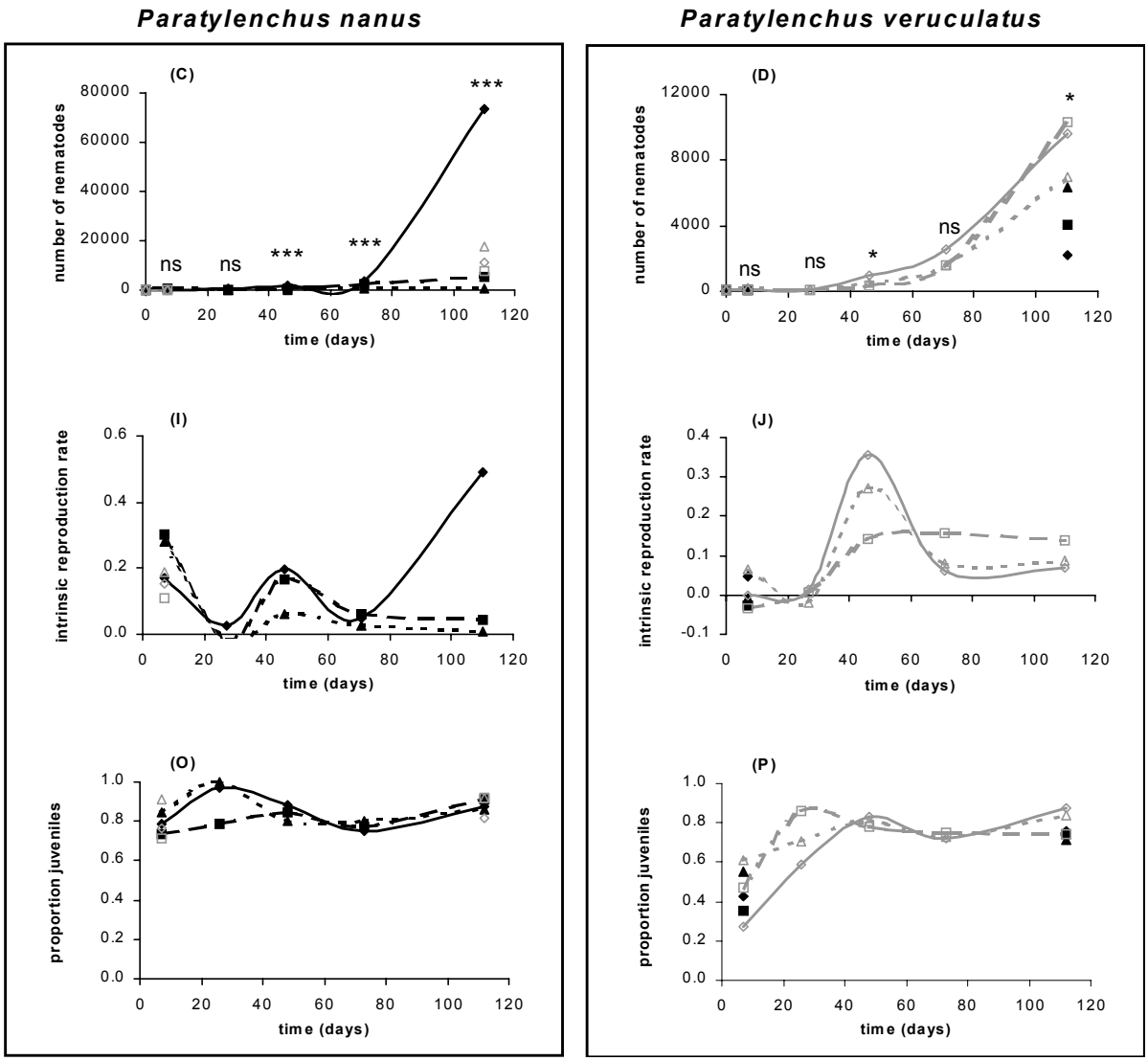


Figure 8.2 continued

H. pseudorobustus increased on all plant species with the highest numbers found in pots with *L. perenne* (Fig 8.2a). The lowest population growth was found on *A. odoratum*, *H. lanatus* and *F. rubra*. The intrinsic reproduction rate was high in the first week of the experiment, decreased in the next weeks and peaked between days 27 and 46 under *H. lanatus* and *A. stolonifera* and between days 46 and 71 under *L. perenne* (Fig 8.2g). At the end of the experiment population growth ceased. A high reproduction rate was generally attended by an increased proportion of juveniles (Fig 8.2m). The proportion of juveniles significantly differed between harvest dates and between plant species (Table 8.1).

At the last harvest date (day 110) a significantly higher number of *G. nanus* was found in pots with *F. rubra,* whereas the lowest numbers were found in pots with *L. perenne*, *H.*

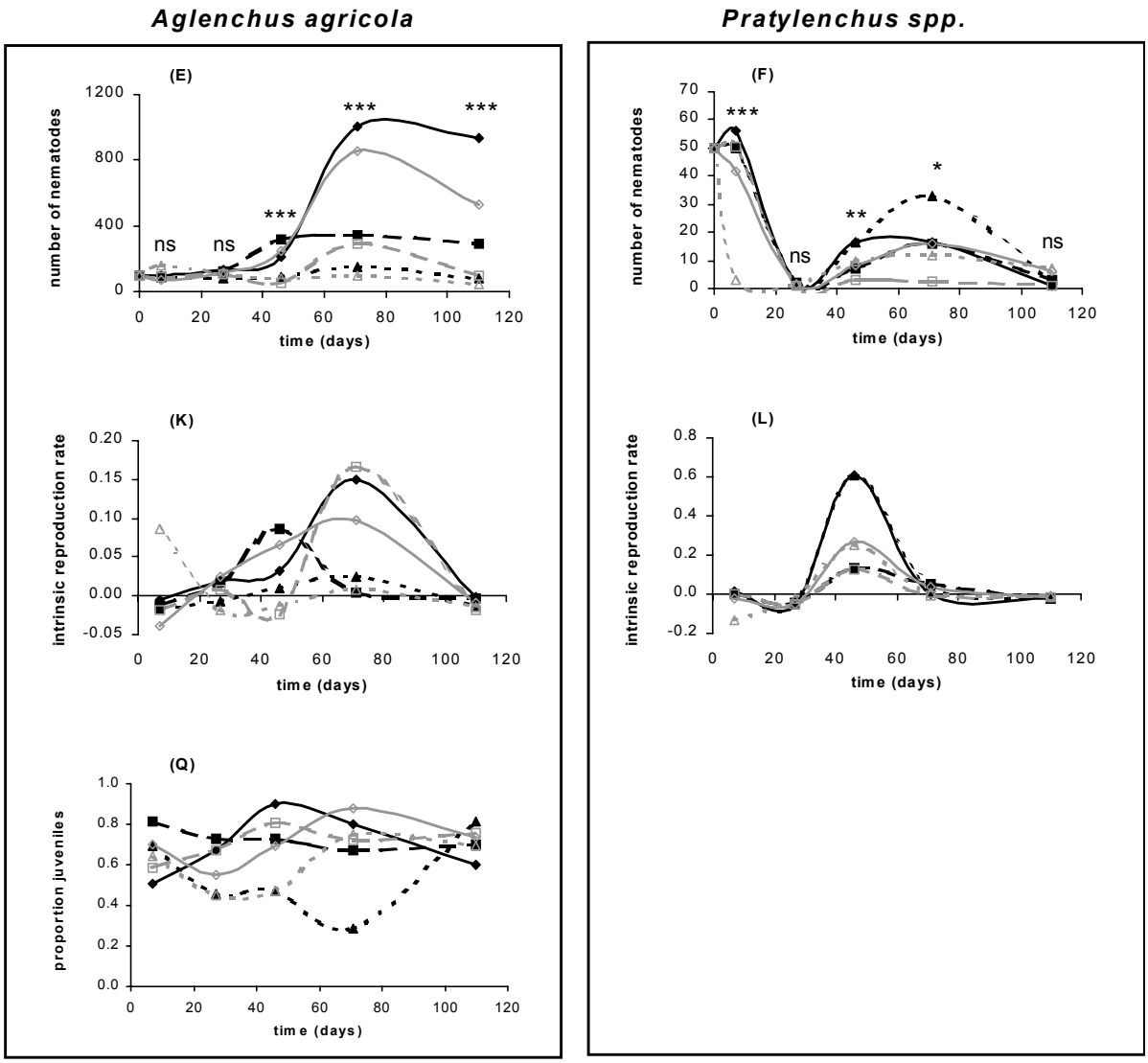


Figure 8.2 continued

lanatus, *A. stolonifera* and *A. capillaris* (Fig 8.2b). Two periods of relatively high intrinsic rates of reproduction were found for *G. nanus*, firstly between days 7 and 27, and secondly between days 46 and 71 (Fig 8.2h). At the end of the experiment population growth ceased. High reproduction rates coincided with high proportions of juveniles (Fig 8.2n)

The populations of both *Paratylenchus* species increased on all plant species, but the host specificity was considerably different for the two nematode species. The highest populations of *P. nanus* developed in the presence of *L. perenne*, whereas the lowest numbers were found on *A. stolonifera* (Fig 8.2c). In contrast, *P. veruculatus* produced the lowest numbers on *L. perenne*, whereas the highest population growth was found in pots with *A. odoratum*, *F.*

rubra and to a lesser extent *A. capillaris* and *A. stolonifera* (Fig 8.2d). The intrinsic reproduction rate of both *Paratylenchus* species peaked between days 27 and 46 (Fig 8.2i,j). Thereafter, the reproduction rate stabilised on a low level, except for an increased reproduction rates of *P. nanus* on *L. perenne*. The proportion of juveniles was significantly affected by date and in case of *P. nanus* also by plant species (Table 8.1). The proportion of juveniles was the highest at day 27 and day 110 (Fig 8.2o,p).

Table 8.1. Results of ANOVA testing the effects of plant species and harvest date on the proportion of juveniles of five nematode species.

	P values				
	H. pseudorobustus	*G. nanus*	*P. nanus*	*P. veruculatus*	*A. agricola*
Plant species	0.048*	0.071	0.047*	0.230	0.585
Date	0.002**	0.000***	0.000***	0.000***	0.758
Plant × Date	0.18	0.217	0.015*	0.011*	0.439

$P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

The numbers of *A. agricola* strongly increased on *L. perenne* and *F. rubra* after day 46, whereas only slight increases were observed on *H. lanatus* and *A. odoratum* (Fig 8.2e). No increase was observed on *A. stolonifera* and *A. capillaris*. At the end of the experiment (day 110), however, the population of *A. agricola* on most plant species slightly decreased again, which resulted in a negative intrinsic reproduction rate (Fig 8.2k). The intrinsic rates and proportions of juveniles fluctuated considerably with time and plant species, but did not result in a statistically significant effect (Table 8.1).

Seven days after inoculation 99% of the inoculated *Pratylenchus* could be recovered (except for only 6% recovery under *A. capillaris*), but on all other harvest dates the recovery of *Pratylenchus* was much lower (Fig 8.2f). After 27 days only 3% of the initial number of *Pratylenchus* was found, but on average 20 and 32% of *Pratylenchus* recovered after 46 and 71 days incubation, respectively. On the last harvest date the number of *Pratylenchus* had decreased again to about 7% of the inoculum. The intrinsic reproduction rate, therefore, peaked between days 27 and 46 (Fig 8.2l). Significant differences in the number of *Pratylenchus* between the plant species were found on day 46 and day 71 only. The highest recovery of *Pratylenchus* was found for *A. stolonifera*, whereas the lowest recovery was

found for *A. odoratum*. We assume that most of the endoparasitic *Pratylenchus* had moved into the roots within a few weeks after inoculation, resulting in an apparent disappearance after 27 days. Although we have tried to extract *Pratylenchus* from the roots as well, we suppose that the extraction method we used was not suitable for *Pratylenchus*, which resulted in the low recovery.

To test whether plant-feeding nematodes could reproduce better on plant species of high-production grasslands than on plant species of low-production grasslands, we compared the nematode numbers that developed after 110 days on species of high-production grasslands (*L. perenne*, *H. lanatus* and *A. stolonifera*) with those on species of low-production grasslands (*F. rubra*, *A. odoratum* and *A. capillaris*). A significant difference between these groups was only found for *P. veruculatus* (Table 8.2). The number of *P. veruculatus*, however, was higher in pots with plant species of low-production grasslands than in pots with species of high-production grasslands.

Table 8.2. Statistical difference (tested by ANOVA) between the number of nematodes per pot at day 110, that developed in the root zones of plant species characteristic of high-production fields (*L. perenne*, *H. lanatus* and *A. stolonifera*) and plant species characteristic of low-production fields (*F. rubra*, *A. odoratum* and *A. capillaris*).

species	df	*F* value	Mean number of nematodes	
			High production plant species	Low production plant species
Pratylenchus spp	1	0.002	2	4
Helicotylenchus pseudorobustus	1	1.553	1050	826
Geocenamus nanus	1	1.660	3746	7200
Paratylenchus nanus	1	0.572	26529	12172
Paratylenchus veruculatus	1	10.821 **	4216	8961
Aglenchus agricola	1	1.959	435	221

** $P \leq 0.01$

Discussion

The decreasing reproduction rates of some nematode species at the end of the experiment might have been caused by a reduced root activity of the host plants. Root activity is an

important factor affecting the reproduction of nematodes (Yeates, 1981). Generally, plant-feeding nematodes prefer the young root tissues such as root tips, meristems and root hairs, as feeding sites. Aging of roots will, therefore, result in reduced numbers of suitable feeding sites and worse conditions for nematode population growth. The reduced reproduction rates of *H. pseudorobustus*, *G. nanus* and *A. agricola* indicate that a maximum carrying capacity was reached before the end of the experiment. Only the reproduction rates of the two *Paratylenchus* species remained relatively high at the end of the experiment in spite of a reduced root growth. Possibly, their small body size in combination with a sedentary feeding strategy might enable these nematodes to maintain a high reproduction also on older root tissues with a lower food quality (chapter 2: Verschoor et al., 2001a).

In contrast to the other plant species, the root growth rate of *F. rubra* increased at the end of the experiment, which might have resulted from the increased fertiliser supply after 71 days. The increased growth rate of *F. rubra*, however, did not result in an increased reproduction rate of nematodes. It is likely, however, that the nematode response to a higher root activity will be delayed and could, therefore, not be noticed within the period of experimentation.

Nevertheless, high reproduction rates were found on plant species with a relatively low root biomass (growth rate) indicating that species-specific interactions between nematodes and plant species exist independent of root biomass. The results of the present study showed that in agreement with our hypothesis, the reproduction rate of nematodes differed considerably between plant species suggesting some level of host specificity of the examined nematode species.

Such host specificity may be affected by the life strategy of the plant species. Plant species from high-production environments generally have a higher nutrient uptake rate, a higher growth rate, a shorter tissue life span and a lower tissue density than species from low-production environments (Ryser, 1996a,b). Since the palatability of plants is negatively correlated with the tissue life span (Southwood et al., 1986; Coley, 1988), it can be expected that plant species from low-production habitats have a lower palatability to herbivores than species of high-production habitats. Such lower palatability of plants will have a direct negative effect on the number of herbivores that feed on these plants. It can be expected, therefore, that when feeding on dominant plant species from low-production grasslands, such as *F. rubra*, *A. odoratum* and *A. capillaris*, the nematode population growth will be lower than when feeding on plant species that dominate in high-production grasslands, such as *L. perenne*, *H. lanatus* and *A. stolonifera*. Indeed, earlier studies presented in this thesis

(chapters 5, 6 and 7) supported this hypothesis in showing a consistently higher number of plant-feeding nematodes under plant species of high-production grasslands. Furthermore, the decreasing numbers of plant-feeding nematodes with time of non-fertilisation in grasslands of the Drentse A nature reserve supports this hypothesis (chapter 2: Verschoor et al., 2001a). The host-specificity results of the present study, however, do not fully support the observations of the fore-mentioned earlier experiments (Table 8.3). The only statistically significant difference between plant species of high-production and low-production grasslands, was found for *P. veruculatus*, which, however, developed better on plant species of low-production grasslands. Also *G. nanus* showed the highest reproduction on two plant species, *F. rubra* and *A. odoratum*, that are representatives of low-production grasslands. So the results of the present study do not confirm our hypothesis that species of high-production grasslands are better hosts for plant-feeding nematodes than species of low-production grasslands.

Several explanations can be found for the contradicting results between the present study and the earlier experiments presented in this thesis (Table 8.3). Firstly, the presence of interspecific competition with other nematode species in the earlier studies and part of the present study (in case of *H. pseudorobustus* and *P. veruculatus*) might have affected the results, as the outcome of competition is probably dependent on the host species. Secondly, the soil and growth conditions were different in each experiment, which might have affected nematode activity and mobility, plant growth and quality, and indirectly nematode-nematode interactions. Particularly, for plant species of high-production sites such as *H. lanatus* and *L. perenne*, the growth conditions in the present study were not optimal due to a low supply of fertilisers. In contrast to the earlier experiments presented in this thesis (Table 8.3), these plant species produced less root biomass than the representatives of low-production sites, *F. rubra* and *A. odoratum*. Although root biomass is not always a good indicator of root productivity, the relatively low root biomass of *H. lanatus* and *L. perenne* might have resulted in a lower number of feeding sites and a subsequent slower development of nematode populations.

As we have found differential effects of plant species on the reproduction rates of the nematode species, it can be expected that the abundance of these nematodes in the field is affected by the composition of the plant community. A nematode survey in hayfields, that were formerly managed according to standard agricultural practice of grasslands, showed that both the composition of the plant and nematode community changed with the number of years that these grasslands remained unfertilised (chapter 2: Verschoor et al., 2001a).

Furthermore, small but significant differences in nematode numbers were found for some species (*H. pseudorobustus*, *G. nanus*, *P. nanus* and *P. veruculatus*) between samples that were taken in the root zones of coexisting plant species within the same grassland (chapter 2: Verschoor et al., 2001a). The results of the present study, however,

Table 8.3. Summary of observed host-specific relationships between nematode taxa and grass species in this study and related studies (chapters 2, 5, 6, and 7). Plant species (*Lolium perenne* (Lp); *Holcus lanatus* (Hl); *Agrostis stolonifera* (As); *Festuca rubra* (Fr); *Anthoxanthum odoratum* (Ao); *Agrostis capillaris* (As)) are given in descending order by the number of nematodes that developed in their root zones. (>) indicates a numerical decrease; (-) is not determined; (ns) indicates no significant difference in nematode numbers between plant species; all other interactions are significant for $P<0.05$.

	Chapter 2	Chapter 5	Chapter 6	Chapter 7	This study
Nematode taxa of high-production grasslands					
Pratylenchus spp.	Ns	Lp>Fr	Ns	(Lp,Hl)>Fr>Ao	-
H. pseudorobustus	Hl>Lp	ns	Hl>Ao	Hl>Lp>(Fr,Ao)	Lp>(As,Ac)>Fr>Hl>Ao
G. nanus	Lp>Hl	Lp>Fr	Hl>Ao	Ns	Fr>Ao>(Lp,Hl,As)>Ac
P. nanus	Hl>Lp	Lp>Fr	Hl>Ao	Hl>Lp>(Fr,Ao)	Lp>(Ac,Fr,Ao,Hl)>As
Tylenchidae/ *A. agricola*	Ns	ns	Ns	(Hl,Lp)>(Fr,Ao)	Lp>Fr>Hl>(Ao,As,Ac)*
Nematode taxa of low-production grasslands					
Pratylenchus spp.	Ns	ns	-	Lp>Hl>Fr>Ao	Ns
H. pseudorobustus	Ns	-	-	Lp>(Hl,Fr)>Ao	-
P. veruculatus	Hl>(Fr,Ao)	Lp>Fr	-	Ao>Hl>Fr>Lp	(Ao,Fr)>(Ac,As)>Hl>Lp
Tylenchidae/ *A. agricola*	(Hl,Ao)>Fr	ns	-	Hl>Lp>(Fr,Ao)	Lp>Fr>Hl>(Ao,As,Ac)*

Investigated plant species:
Chapter 2 (field survey): Lp and Hl in high-production and Hl, Fr and Ao in low-production grasslands
Chapter 5 (inoculation experiment): Lp and Fr
Chapter 6 (competition experiment): Hl and Ao
Chapter 7 (field experiment): Lp, Hl, Fr and Ao.
* note: the results of *A. agricola* are presented both as nematodes of low-production and as nematodes of high-production grasslands.

were generally not in agreement with the observations in the field. Firstly, the differences in the mean numbers of nematodes between root zones of coexisting plant species were practically opposite to the reproduction results in the present study (Table 8.3). Secondly, nematode species were generally not more abundant in successional stages that were dominated by the plant species on which the highest reproduction rates were found. For example, the highest numbers of *G. nanus*, which is strictly found in the early-successional high-production sites, were found on plant species of the late-successional low-production sites. Only the high reproduction rates of *P. veruculatus* on plant species of low-production grasslands (*F. rubra*, *A. odoratum* and *A. capillaris*), were in agreement with its prevalence in the low-production grasslands, which might indicate a direct relationship between the distributions of nematode and host species.

On the other hand the absence of *P. veruculatus* in the high-production grasslands could not be explained by a relative low reproduction on the dominant plant species of the high-production grasslands only, as the absolute reproduction rate on these plant species was still very high compared to other nematode species. Also Wood (1973) concluded that because of the wide host range of *Paratylenchus projectus* the distribution pattern of plant species is unlikely to have a direct influence on the distribution pattern of *P. projectus* in pastures. Apparently, other biotic or abiotic factors affected its occurrence as well. Competition with other nematode species, particularly species with similar feeding habits, might be one of these factors (Eisenback, 1993). An interesting example of competition between nematode species, occupying similar ecological niches, is given by Boag and Alphey (1988). They found that the numbers of *P. nanus* rapidly increased after the numbers of the coexisting *Rotylenchus robustus*, *Trichodorus primitivus* and *Paratrichodorus pachydermus* were reduced following treatment with dichloropropene. In our study area, *P. nanus*, which is a dominant species in the high-production grasslands, might have been a direct competitor of the related *P. veruculatus*. Changes in the environmental conditions can modify the outcome of nematode-nematode interactions (Eisenback, 1993). In this respect, it is not surprising that these two nematode species could only coexist in a transitional stage of succession between a high-production and a low-production grassland, that was still dominated by plant species (mainly *H. lanatus*) of high-production grasslands (chapter 2: Verschoor et al., 2001a).

Interpretation of the species-specific reproduction rates of nematodes in terms of their ecological significance for spatio-temporal vegetation dynamics is complicated. Firstly, the results of experiments, as presented in this paper, are dependent on the modifying effects of (a)biotic factors on nematode-plant and nematode-nematode interactions. Secondly, it gives

no information on the sensitivity of plant species for different nematode species. Some plant species can tolerate high numbers of nematodes without any appreciable damage to the plant, whereas the growth of other plant species can be reduced already in the presence of low numbers of the same nematode species (Van der Stoel and Van der Putten, unpublished data). To study the role of host specificity of plant-feeding nematodes for spatio-temporal vegetation dynamics in grasslands we, therefore, need further investigations on the density-dependent effects of nematodes on plant growth under different growth conditions. The present study demonstrates, however, that spatio-temporal distributions of plant-feeding nematodes do not mainly depend on the presence of host plant species, but must to a large extent depend, directly or indirectly, on other factors such as interspecific competition and abiotic conditions.

9. General discussion

Management aimed at reduction of the nutrient availability in the soil will sooner or later result in a replacement of light-competing plant species by plant species that are better competitors for nutrients. Since the relative contribution of soil organisms to ecosystem processes such as nutrient cycling and primary production increases after fertilisation is stopped (Brussaard et al., 1996 ; Brussaard, 1998), policy makers have to be aware of the consequences of restoration management on biotic soil processes. In this thesis I have tried to get insight into the effects of restoration management on the interactions between plants and plant-feeding nematodes.

HOW PLANTS AFFECT NEMATODE SUCCESSION

The reduction of nutrient availability in the grasslands of the Drentse A nature reserve resulted in a gradual change of both the plant and plant-feeding nematode community (chapter 2). Nematological research in agricultural systems has shown that most plant-feeding nematode species have evolved a high level of host specificity for plant species. It could be expected, therefore, that the succession of nematode species would reflect the succession of plant species. I found, however, only poor evidence for such species-specific interactions in the grasslands of the Drentse A nature reserve. A nematode survey under different plant species within these grasslands showed only small differences in the composition of the plant-feeding nematode communities between the plant species (chapter 2). Furthermore, nematode species showed specific population growth rates on different plant species, but generally there was no clear-cut relationship between the abundance of each nematode species in the field and the presence of host plant species (chapter 8).

The results of the nematode survey (chapter 2) indicated that the succession of plant-feeding nematodes with time of non-fertilisation was more affected by qualitative changes within the plant species than by the species composition of the vegetation. Particularly, a

decrease of nutrient concentrations in the plant tissues with time of non-fertilisation (Olff, 1992a) might have affected the composition of the plant-feeding nematode community. In line with these results and our hypotheses, these qualitative changes in the plant community with time of non-fertilisation resulted in a lower density and total biomass of plant-feeding nematodes and a replacement of nematode species having low nutrient-use efficiencies by species having high nutrient-use efficiencies.

The close relationship between the plant nutrient composition and the composition and abundance of the nematode community is supported by results of the field experiment (chapter 7). For example, alterations of the N concentrations in the root tissues after fertilisation and liming were accompanied by alterations in the nematode community. Generally, the numbers of plant-feeding nematodes increased at higher N concentrations, which is in agreement with the findings of other studies (Sohlenius and Boström, 1986; Yeates, 1987; Todd, 1996). On the other hand no relationship could be found between the root N concentrations of different plant species and the numbers of plant-feeding nematodes which were found belowground (chapters 5, 6, and 7). Relationships between total plant N and herbivore performance are, however, not clear-cut, since the total N may not always be a good indicator of the available N (Curry, 1994). Particularly for sap-sucking herbivores such as nematodes, only a part of the total N is available for consumption. Furthermore, plant species differ in their physical/chemical defence against herbivores irrespective of their tissue N concentrations.

Besides the nutrient status of the plant, other characteristics of plant species can be important as determinants of nematode density and species composition. Plant species of nutrient-rich habitats generally have a higher nutrient uptake rate, a higher growth rate, a shorter tissue life expectancy and a lower tissue density than species of nutrient-poor habitats (Ryser, 1996a,b). Numbers of nematodes are in general positively correlated with the productivity of the vegetation (Yeates, 1987). In both the field survey (chapter 2) and in the experimental studies (chapters 5, 6, and 7), I found no relationship between the standing root biomass and the numbers of nematodes. Standing root biomass, however, may not be a good indicator of plant productivity because the turnover rate of the plant tissues can also be positively related to plant productivity. Since the palatability of plants is negatively correlated with the life expectancy of the plant organs (Southwood et al., 1986), a high palatability rather than a high root biomass might have caused the high numbers and total biomass of plant-feeding nematodes in the nutrient-rich grasslands. Such a high palatability of fast-growing plant species can be the result of a higher content of available N (Mattson, 1980) or

a lower chemical defence (Edwards-Jones and Brown, 1993). Furthermore, the higher tissue density of slow-growing species can be a mechanical defence mechanism that hinders the feeding of nematodes.

I hypothesised, therefore, that a higher reproduction rate of plant-feeding nematodes will be found under plant-species from high-production habitats. In line with this hypothesis, I found, both in greenhouse and field experiments, higher numbers of plant-feeding nematodes under plant species from high-production habitats than under species of low-production habitats (chapters 5, 6, and 7). These results, however, were not supported by the results of the host specificity experiment (chapter 8) in which no relationship between nematode reproduction rate and habitat preference of the plant species were found. Neither did I find a relationship between the reproduction rates of nematodes and the growth rates of the plant species, irrespective of their habitat preference. Since all plants in the host specificity experiment were grown under similar growth conditions, this experiment gave no information on the nematode reproduction rates under different growth conditions. Fertilisation of field plots in the low-production grasslands, however, caused a higher productivity of the vegetation and considerable increases in plant-feeding nematodes, whereas no such effect was found in the nutrient-rich high-production grasslands (chapter 7). These results, therefore, confirmed that qualitative differences within plant species rather than qualitative differences between plant species affected the succession of plant-feeding nematodes.

HOW NEMATODES AFFECT PLANT SUCCESSION

Under optimal growth conditions plants can tolerate high numbers of plant-feeding nematodes because their high growth rate offsets the damage caused by nematodes (Yeates, 1987). This situation may change, however, when the growth conditions deteriorate at decreasing nutrient levels. White (1984) reviewed the literature on the relationship between the abundance of herbivores and the availability of nitrogen in stressed food plants. When plants are stressed, for example by nutrient deficiencies, the soluble nitrogen content of the plant increases. Such soluble nitrogen is an adequate source of food for young herbivores and an increase of its concentration in the plant, therefore, can stimulate the numbers of herbivores. Furthermore, plant productivity decreases at lower nutrient supplies. Hence, herbivore damage is potentially higher at decreasing nitrogen levels (Brussaard, 1998). I

hypothesised, therefore, that when the nutrient availability decreases in the studied grasslands after the cessation of fertilisation, nematode damage to the plants would increase.

Estimations of plant consumption by plant-feeding nematodes based on data of the field survey did not support a negative relationship between the nutrient availability in the soil and potential nematode damage (chapter 4). The proportion of the standing root biomass that was consumed by nematodes was more or less similar in all the studied grasslands irrespective of their productivity level. With respect to individual plant species, however, these values are of limited value because the vegetation in each grassland generally consisted of species that are well-adapted to the nutrient-conditions in that site. Experimental studies in which plant species were grown at different nutrient supplies are more informative. *Holcus lanatus*, which is a characteristic plant species of high-production habitats was both more sensitive to nutrient limitation and nematode herbivory than *Anthoxanthum odoratum*, a species of low-production habitats (chapter 6). I found no indication, however, that *H. lanatus* was more affected by plant-feeding nematodes at low nutrient supplies. In the inoculation experiment (chapter 5) and the field experiment (chapter 7), both nematode inoculation and nematicide treatment had no significant effects on plant productivity. No support for a synergistic effect of nematode herbivory and nutrient stress was, therefore, found in the experimental studies. On the contrary, fertilisation of a low-production site significantly increased the numbers of nematodes and, consequently, increased the damage caused by nematodes to the vegetation (chapter 7).

White (1984) also mentioned such conflicting results with the 'herbivore-stress hypothesis'of increasing herbivore numbers after fertilisation. It would seem that the herbivore densities decrease either below or above a certain optimal N concentration in the tissues of plants. Apparently, the low nutrient supplies in the experimental studies resulted in such low concentrations of soluble N, so that no increase of nematode abundance and damage caused by nematodes occurred.

Since I found no indications that nutrient stress can increase damage caused by nematodes it is questionable whether and how plant-feeding nematodes can affect the vegetation succession. The results of the competition experiment (chapter 6) gave at least promising results showing that plant-feeding nematodes can affect the competition between an early- and late-successional plant species in favour of the latter. The development of species-specific pathogen complexes under each plant species might be an important mechanism of plant succession. No clear-cut indications of species-specific interactions between plant-feeding nematodes and the dominant plant species in the studied grasslands, however, were

found in the experiments. It was suggested, therefore, that in addition to progressing nutrient stress, plant species-specific differences in tolerance, rather than the host specificity of nematodes, may determine the plant species replacement in grasslands under restoration management (chapter 6). On the other hand, plant-feeding nematodes may act as key species in pathogen complexes by creating entries in roots which can stimulate the colonisation of roots by pathogenic micro-organisms (De Rooij-van der Goes, 1995; Van der Putten and Van der Stoel, 1998). In such a way even generalist nematodes can indirectly contribute to species-specific suppression of plant species. At least some of the results in chapters 6 and 7 might have been caused by species-specific suppression of plant growth. For example, the elimination of species-specific nematode communities after nematicide application might have caused the strong increase in the root biomass of *Festuca rubra* in a *Festuca*-dominated site (chapter 7), and the stronger increase in biomass of *Holcus lanatus* compared to *Anthoxanthum odoratum* in soil of a *Holcus*-dominated site (chapter 6).

An important aspect that has generally been overlooked in studies on the relationship between herbivores and plant succession, is the spatio-temporal distribution of herbivores. Within a grassland, large spatial and temporal differences in the numbers of plant-feeding nematodes are present (chapters 2 and 3). Such spatio-temporal variation in nematode abundance approximately corresponded to local consumption values of 1-40% of the root standing biomass (chapter 4). Local hotspots of plant-feeding nematodes might, therefore, cause small-scale die-off in plants. Consequently, new niches are created that will presumably be colonised by plant species that are less sensitive to the nematodes present. These results also imply that by using average numbers of nematodes in experimental studies such as in this thesis, the effects of plant-feeding nematodes on plant growth might have been underestimated.

Another aspect which has not been studied in the present thesis is the impact of nematode herbivory on seedling colonisation. In permanent grasslands the impact of nematode herbivory on the colonisation and establishment of plant seedlings in the vegetation might be of particular importance. Studies on root-feeding insects, for example, have shown that below-ground herbivory can increase seedling mortality and consequently, affect plant diversity and succession (Brown and Gange, 1989, 1991, 1992; Ganada and Brown, 1997). The nitrogen-rich seedlings are attractive food sources for young nematodes (Mattson, 1980) and are particularly vulnerable to infestation by nematodes or other pathogens (Curry, 1994; Marschner, 1995). The presence of plant-feeding nematodes might, therefore, decrease seedling survival and subsequently affect the species composition of the plant community.

On the other hand, established plants are less sensitive to damage caused by nematodes than seedlings and have the opportunity to reproduce vegetatively. Additional research is, therefore, needed on the effects of nematode herbivory on the settlement of seedlings within established vegetation.

The effects of plant-feeding nematodes on the rate of plant succession were not clear-cut. The suppression of the early-successional *H. lanatus* by plant-feeding nematodes in favour of the late-successional *A. odoratum* suggests that nematode herbivory might speed up plant succession in grasslands after fertilisation is stopped. Such an acceleration of succession could be caused both by the synergistic effects of plant-feeding nematodes and nutrient stress, and by species-specific suppression of plant species. Plant-feeding nematodes, therefore, are potentially an important biotic factor in the succession of plant communities, whose activity can help to restore the former species-rich plant communities of nutrient-poor habitats. On the other hand gradual changes in the nutritional quality of the food reduced the numbers and biomass of plant-feeding nematodes with time of non-fertilisation and, consequently, this reduced their absolute contribution to ecosystem processes such as nutrient cycling and primary production (chapter 4). This is supported by the absence of a stimulating effect of nematicide application on plant productivity in the fields. Additional research is needed, therefore, to find out whether plant-feeding nematodes are directors or followers of plant succession. The results in this thesis so far indicate that the succession of plant-feeding nematodes is more affected by the successional changes in the plant community than the other way round.

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Summary

Interactions between plants and herbivores can be of critical importance to the course of succession. Plant-feeding nematodes are among the most dominant groups of herbivores and are considered to be an important constraint of primary production in grasslands. In this thesis the results are presented of a study on the interactions between plants and plant-feeding nematodes in grasslands that are under restoration management.

The studied grasslands were formerly used for agricultural practice and fertilisers were applied to facilitate high productivity. In order to restore the former species-rich plant communities, the Dutch State Forestry Commission applies a management strategy of ceasing the application of fertilisers while nutrients are removed by haymaking. Such management has resulted in a decrease in the nutrient availability in the soil and, subsequently, succession of plants in which species characteristic of high-productive habitats are replaced by species characteristic of low-productive habitats.

It was hypothesised that root herbivory by plant-feeding nematodes affects the competition between early- and late-successional plant species in favour of the latter, leading to an acceleration of plant succession. Such suppression of early-successional plant species by late-successional species can be caused by two mechanisms. Firstly, early-successional plant species are more sensitive to nutrient-poor conditions than late-successional species. Such an increasing stress due to lower nutrient supplies is assumed to cause a higher sensitivity to nematode infestation and, subsequently, lower competitive abilities of the early-successional species. Secondly, species-specific nematode communities can develop under the early-successional species, which may affect the growth of these plant species, but not that of their successors. Furthermore, it was hypothesised that a reduction in nutrient supply will result in a lower food quality for plant-feeding nematodes. Subsequently, the abundance of plant-feeding nematodes will decrease and nematode species with low nutrient-use efficiencies will be replaced by species with high nutrient-use efficiencies.

The community structure and abundance of plant-feeding nematodes were studied in four grasslands, which had not been fertilised for 6, 10, 23, and 28 years, respectively (chapter 2). Gradual changes in the nematode fauna after the cessation of fertiliser application were found. Sampling under different plant species showed that only minor differences in the species composition of the plant-feeding nematode community were present between plant

species within each site, whereas major changes were found under single plant species between the sites. These results indicate that qualitative changes within a plant species rather than qualitative differences between plant species affected the nematode community. In agreement with my expectations, the numbers and biomass of plant-feeding nematodes decreased with time of non-fertilisation. Furthermore, it seemed that a reduction in the nutrient availability selected for nematode species that were better adapted to conditions of low food quality. Particularly the average nematode body size, which is constrained by the absolute amount of food necessary for growth and maintenance, decreased at a lower nutrient availability. Feeding strategies, however, that reduce the energetic costs of feeding and movement, such as endoparasitism, a longer stylet, and the modification of feeding cells, seemed to enable certain nematode species to support a relatively large body size also at a low nutrient availability.

The seasonal changes in the composition of the plant-feeding nematode community were generally small (chapter 2). Nevertheless, most nematode taxa had a specific annual cycle in their abundance and population structures, which could largely be related to seasonal changes in the temperature and moisture contents of the soil (chapter 3). The spatial distribution of plant-feeding nematodes along a 40 cm soil profile was also investigated (chapter 3). Plant-feeding nematode species differed considerably in their vertical distribution. The density of most nematode species was highest in the upper 10-cm soil layer and strongly decreased at greater depth. Their vertical distribution was correlated with the distribution of roots in the soil profile. Some nematode taxa such as Trichodoridae and *Hemicycliophora*, however, were more prevalent in the deeper soil layers.

Based on these survey results (chapters 2 and 3), the amounts of carbon and nitrogen that are transferred from the plant biomass to the soil by plant-feeding nematodes were calculated for each grasslands studied (chapter 4). The decrease in nematode biomass with time of non-fertilisation coincided with a decrease in the total CO_2 respiration rate of plant-feeding nematodes from 4.5 l CO_2 m^{-2} $year^{-1}$ (after 6 years without fertilisation) to 1.7 l CO_2 m^{-2} $year^{-1}$ (after 28 years without fertilisation). This corresponded to a carbon consumption of 15.95 to 6.13 g C m^{-2} $year^{-1}$ and a nitrogen consumption of 2.13 to 0.82 g N m^{-2} $year^{-1}$, respectively. The amounts of N mineralised by the plant-feeding nematodes corresponded to approximately 2-5% of the total N mineralisation. The indirect contribution of plant-feeding nematodes to the total N mineralisation, however, may be much higher due to the release of relatively high amounts of organic nitrogen to the soil by defecation, biomass turnover and increased root exudation. The biomass consumption of plant-feeding nematodes was

approximately 3-5% of the total standing biomass and 5-9% of the root standing biomass at each grassland site. Within a grassland site, however, large local differences in the numbers of plant-feeding nematodes resulted in consumption values of 1-40% of the root standing biomass. This spatial heterogeneity in nematode consumption implies that locally, high levels of herbivory can be present which may have considerable effects on plant growth and competition.

The hypothesis that under poor nutrient conditions, an early-successional plant species is more sensitive to nematode infestation than a late-successional plant species, was tested by two greenhouse experiments. In the first experiment, plants of the early-successional *Lolium perenne* and the late-successional *Festuca rubra* were grown in sterilised soil both with and without nematodes (chapter 5). The nematodes were isolated from both an early and a late-successional site to test whether species-specific nematode communities were present in the soils that might cause species-specific suppression of plant growth. In the second greenhouse experiment, plants of the early- successional *Holcus lanatus* and late-successional *Anthoxanthum odoratum* were grown on unsterilised soil of an early-successional field (chapter 6). Half of the pots was treated with nematicide to eliminate the nematodes. Furthermore, both plant species were grown both in monoculture and in mixed culture at different plant densities to investigate the effects of plant-feeding nematodes on the competition between both species. Both greenhouse experiments were performed at low and high supply rates of nutrients. In the first experiment, the addition of nematodes did not affect plant biomass. This might have been caused by a relatively low initial density of plant-feeding nematodes in the pots compared to the densities in the field. In the second experiment, however, an average field density of nematodes was present. In agreement with our expectations, *H. lanatus* was more sensitive to plant-feeding nematodes and nutrient limitation than *A. odoratum*. Therefore, plant-feeding nematodes and low nutrient supplies reduced the competitive abilities of *H. lanatus* in favour of *A. odoratum*. A low nutrient supply, however, did not enhance the effect of plant-feeding nematodes on plant growth and competition, indicating an additive and not a synergistic effect of nematodes and nutrient limitation on plant performance.

The effects of plant-feeding nematodes on plant productivity were also tested in a field experiment (chapter 7). In this experiment the nematode numbers, nutrient availability, and soil pH were manipulated in small field plots by the application of nematicide, NPK fertilisers and lime, respectively. Furthermore, the effects of these treatments on plant productivity were investigated on four grass species, grown in plastic tubes, that were

experimentally introduced to the fields. Nematicide treatment had no main effect on vegetation biomass, although it reduced plant-feeding nematode numbers by 70-85%. The only increase in plant biomass after nematicide treatment (+72%) was found in fertilised plots of a late-successional field. In this field, fertilisation considerably increased the numbers of plant-feeding nematodes, which apparently resulted in a strong reduction of plant growth by these nematodes. This result is in contrast to our expectations that fertilisation might offset damage caused by nematodes due to higher root biomass production and, subsequently, a lower density of nematodes per gram of root. Plant sensitivity to nematode infestation is, therefore, not per se higher at nutrient-poor conditions. Furthermore, the average root biomass of the late-successional species *F. rubra* when grown in a low-productive field increased (+67%) after nematicide application, which might indicate some species-specific suppression of plant growth.

In all three experiments (chapters 5, 6 and 7), the density of plant- and fungal-feeding nematodes was strongly affected by the different treatments. In the greenhouse experiments and in the planted tubes in the field, nematode numbers were generally reduced by fertilisation. This is probably caused by a direct toxic effect of the added nutrient solution. In the field plots, however, fertilisation reduced the numbers of plant-feeding nematodes in the short term, but in the long term the numbers of nematodes increased. Liming generally reduced the numbers of plant-feeding nematodes. Since root N concentrations increased after fertilisation and decreased after liming, I suppose that the numbers of plant-feeding nematodes were particularly affected by the root N concentration. These results support the conclusion that qualitative changes within plant species strongly affected the succession of plant-feeding nematodes with time of non-fertilisation.

The numbers of plant-feeding nematodes also differed between the plant species. In all three experiments higher numbers of plant-feeding nematodes were found under plant species of early-successional habitats (chapters 5, 6 and 7). In a host specificity experiment, however, no relationship was found between the reproduction rate of six different nematode species under six plant species, characteristic of different habitats, and the habitat preference of the host plant (chapter 8). Furthermore, we found no relationship between the reproduction rate of nematodes on different plant species and the coexistence of nematode and plant species in the field. Thus, nematode species were not necessarily more abundant in fields dominated by plant species on which high reproduction rates were found. We concluded, therefore, that spatio-temporal distributions of plant-feeding nematodes do not mainly depend on the

presence of host plant species, but must to a large extent depend on other factors such as interspecific competition and abiotic conditions.

Estimations of the biomass consumption by plant-feeding nematodes (chapter 4) and their effects on the competition between plant species (chapter 6) showed that plant-feeding nematodes are potentially an important biotic factor in the succession of plant communities. Based on the results of this thesis, however, no unequivocal conclusions can be drawn yet about their actual impact on the succession in grasslands under restoration management. The decreasing numbers and biomass of plant-feeding nematodes with time of non-fertilisation also resulted in a lower absolute contribution of plant-feeding nematodes to ecosystem processes such as nutrient cycling and primary production (chapter 4). This is supported by the absence of a stimulating effect of nematicide application on plant productivity in the fields (chapter 7). Additional research is needed, therefore, to find out whether plant-feeding nematodes can affect plant succession in the grasslands of the Drentse A nature reserve. So far the results presented in this thesis suggest that the succession of the plant-feeding nematode community is probably more affected by changes in the plant community than the other way round.

Samenvatting

Herbivoren kunnen een belangrijke invloed uitoefenen op de ontwikkeling van vegetatie (vegetatiesuccessie). Plantenparasitaire nematoden behoren tot de meest dominante groep van herbivoren in ecosystemen en worden gezien als een belangrijke factor die de productiviteit van graslanden kan beperken. In dit proefschrift worden de resultaten gepresenteerd van een onderzoek naar de interacties tussen planten en plantenparasitaire nematoden in een successiereeks van hoog naar laag productieve graslanden.

De onderzochte graslanden zijn gelegen in het stroomdal van de Drentse A en werden voorheen gebruikt als hooiland of werden extensief begraasd door vee. Om de productiveit van deze graslanden te verhogen is mest toegediend, waardoor de vroegere soortenrijke plantengemeenschappen van arme hooilanden zijn verdwenen. Om de oude situatie te herstellen heeft Staatsbosbeheer deze graslanden opgekocht, de bemesting stopgezet en jaarlijks gemaaid. Dit verschralingbeheer heeft geresulteerd in een afname van de voedselbeschikbaarheid voor planten en een vegetatiesuccessie, waarbij plantensoorten van hoogproductieve omstandigheden (vroege successiesoorten) werden vervangen door soorten van laagproductieve omstandigheden (late successiesoorten).

De verwachting is nu dat wortelvraat door nematoden deze successie kan beïnvloeden doordat het de concurrentie tussen vroege en late successiesoorten kan beïnvloeden ten gunste van de laatste. De grotere gevoeligheid voor nematoden van een vroege ten opzichte van een late successiesoort kan twee oorzaken hebben. In de eerste plaats zijn vroege successiesoorten gevoeliger voor een lage beschikbaarheid aan voedingsstoffen dan late successiesoorten. Een toenemende stress ten gevolge van een afnemende voedselbeschikbaarheid kan bij vroege successiesoorten weer resulteren in een grotere gevoeligheid voor nematodenaantasting. In de tweede plaats kunnen onder plantensoorten specifieke nematodengemeenschappen tot ontwikkeling komen die de betreffende (vroege successie-) soorten wel, maar hun opvolgers (de late successiesoorten) niet of minder aantasten. Uiteindelijk kan dit leiden tot een versnelling van de vegetatie-successie. Daarnaast is de verwachting dat een afname in de voedselbeschikbaarheid zal resulteren in een lagere voedselkwaliteit voor plantenparasitaire nematoden. Als gevolg daarvan zal het aantal en de totale biomassa van plantenparasitaire nematoden afnemen en zal een

verschuiving plaatsvinden van soorten met een laag efficiënte opname van voedingsstoffen naar soorten met een hoog efficiënte opname van voedingsstoffen.

De soortensamenstelling en dichtheid aan plantenparasitaire nematoden zijn onderzocht in vier graslanden, die niet bemest zijn geweest gedurende respectievelijk 6, 10, 23 en 28 jaar (hoofdstuk 2). Uit dit onderzoek is gebleken dat een geleidelijke verandering in de samenstelling van de nematodengemeenschap heeft plaatsgevonden na het stopzetten van bemesting. Bemonstering onder verschillende plantensoorten toonde aan dat er slechts kleine verschillen bestonden in de samenstelling van de nematodengemeenschappen tussen plantensoorten binnen een veld. Echter onder één plantensoort, die in verschillende velden is bemonsterd, werden grote verschillen waargenomen in de samenstelling van de nematodengemeenschappen tussen de velden. Deze resultaten laten zien dat het eerder kwalitatieve veranderingen binnen een plantensoort zijn dan veranderingen in de soortensamenstelling van de vegetatie die de successie van plantenparasitaire nematoden bepalen. In overeenstemming met mijn verwachtingen nam het aantal en de totale biomassa van plantenparasitaire nematoden af bij een lagere voedselbeschikbaarheid. Bovendien nam het aandeel nematodensoorten die beter aangepast lijken te zijn aan een lage voedselkwaliteit toe. Met name de gemiddelde lichaamsgrootte van nematoden, die sterk afhangt van de absolute hoeveelheid voedsel die beschikbaar is voor groei en onderhoud, nam sterk af bij een lagere voedselbeschikbaarheid. Echter, voedingsstrategieën die de energetische kosten van eten en bewegen beperken, zoals endoparasitisme, een langere stekel, of de vorming van gespecialiseerde voedingscellen, lijken nematoden in staat te stellen toch een relatief groot lichaam te onderhouden bij een lage voedselbeschikbaarheid.

Gedurende het seizoen zijn slechts geringe veranderingen waargenomen in de samenstelling van de plantenparasitaire nematodengemeenschap (hoofdstuk 2). Desalniettemin vertoonden de meeste nematodensoorten wel een soortspecifieke populatieverloop gedurende het jaar, die grotendeels gerelateerd kon worden aan seizoensveranderingen in de temperatuur en het vochtgehalte van de bodem (hoofdstuk 3). Daarnaast is gebleken dat nematodensoorten sterk verschillen in hun verticale verdeling (hoofdstuk 3). De dichtheid van de meeste soorten was het hoogst in de bovenste 10 cm van de bodem, waarna deze sterk afnam met de diepte. De verticale verdeling van deze soorten was sterk gecorreleerd met de verdeling van de wortels in de grond. Een aantal nematodengroepen, zoals Trichodoridae en *Hemicycliophora*, gaf echter los van de aanwezigheid van wortels de voorkeur aan diepere bodemlagen.

Deze gegevens over de populaties van plantenparasitaire nematoden in ruimte en tijd (hoofdstuk 2 en 3) maakte het mogelijk om de bijdrage van plantenparasitaire nematoden aan de kringloop van voedingstoffen, in dit geval koolstof (C) en stikstof (N), te kwantificeren voor elk van de vier onderzochte graslanden (hoofdstuk 4). De afname in de totale biomassa van plantenparasitaire nematoden gedurende de successie ging gepaard met een afname van de totale hoeveelheid kooldioxide (CO_2) die door de nematoden werd gerespireerd. De CO_2 respiratie liep terug van 4,5 l CO_2 m^{-2} $jaar^{-1}$ na 6 jaar zonder bemesting tot 1,7 l CO_2 m^{-2} $jaar^{-1}$ na 28 jaar zonder bemesting. Dit kwam overeen met een C consumptie door nematoden van respectievelijk 15,9 en 6,1 g C m^{-2} $jaar^{-1}$ en een N consumptie van respectievelijk 2,1 en 0,8 g N m^{-2} $jaar^{-1}$. De hoeveelheid N die door plantenparasitaire nematoden werd gemineraliseerd (dwz. omgezet naar een vorm die weer opgenomen kan worden door planten) komt daarmee ongeveer overeen met 2-5% van de totale N mineralisatie in de grond. De indirecte bijdrage van plantenparasitaire nematoden aan de totale N mineralisatie kan echter veel groter zijn door het vrijkomen van relatief hoge hoeveelheden organisch gebonden N in de grond via faeces, nematodensterfte en de stimulatie van worteluitscheidingen. De gemiddelde consumptie van plantenbiomassa door nematoden was ongeveer 3-5% van de totale plantenbiomassa en 5-9% van de totale wortelbiomassa in elk veld. Echter, grote lokale verschillen in het aantal nematoden binnen een veld resulteerden in consumptiewaarden die uiteen liepen van 1% van de wortelbiomassa bij lage nematodendichtheden tot 40% bij hoge dichtheden. Deze ruimtelijke heterogeniteit in plantconsumptie door nematoden betekent dat plaatselijk aanzienlijke nematodeneffecten op de plantengroei en concurrentie tussen planten te verwachten is.

De hypothese dat bij een lage voedselbeschikbaarheid een vroege successiesoort gevoeliger is voor nematodenaantasting dan een late successiesoort is getoetst aan de hand van twee kasexperimenten. In het eerste kasexperiment werden planten van de vroege successiesoort *Lolium perenne* (Engels raaigras) en de late successiesoort *Festuca rubra* (Rood zwenkgras) opgekweekt op steriele grond zowel met als zonder toevoeging van nematodensuspensies (hoofdstuk 5). Deze nematoden waren geïsoleerd uit grond van zowel een vroeg als een laat successiestadium, om te kunnen toetsen of soortspecifieke nematodengemeenschappen aanwezig waren in de grond. Deze soortspecifieke nematodengemeenschappen zouden de plantensoort afkomstig uit hetzelfde successiestadium meer kunnen aantasten dan de andere plantensoort. In het tweede experiment werden planten van de vroege successiesoort *Holcus lanatus* (Gestreepte witbol) en de late successiesoort *Anthoxanthum odoratum* (Gewoon reukgras) opgekweekt op niet-steriele grond afkomstig uit

een vroeg successiestadium (hoofdstuk 6). Echter nu werden in de helft van de potten de nematoden uitgeschakeld door de toevoeging van nematicide. Bovendien werden beide planten zowel in mono- als in mengcultuur bij verschillende dichtheden opgekweekt, zodat de effecten van nematoden op de concurrentie tussen beide plantensoorten konden worden onderzocht. In beide kasexperimenten werden planten zowel bij een hoog als een laag bemestingsniveau opgekweekt. De toevoeging van nematodensuspensies aan de grond bleek echter geen effect te hebben op de productiviteit van beide plantensoorten. Dit zou veroorzaakt kunnen zijn doordat de nematodendichtheden in de potten lager waren dan de dichtheden in het veld. In het tweede experiment werd echter bij een gemiddelde velddichtheid gewerkt. Nu bleek, in overeenstemming met mijn verwachtingen, de vroege successiesoort *H. lanatus* wel een sterkere concurrent te zijn dan de late successiesoort *A. odoratum*, maar ook sterker te reageren (door een hogere biomassavorming) op bemesting en op de uitschakeling van nematoden dan *A. odoratum*. Daaruit blijkt dat een lage voedselbeschikbaarheid en de aanwezigheid van plantenparasitaire nematoden de concurrentiekracht van de vroege successiesoort *H. lanatus* verminderen ten gunste van de late successiesoort *A. odoratum*. Ik vond echter geen aanwijzingen dat *H. lanatus* bij een lage voedselbeschikbaarheid gevoeliger was voor nematodenaantasting.

De effecten van plantenparasitaire nematoden op de productiviteit van planten werd ook getoetst in een veldexperiment (hoofdstuk 7). In dit experiment werden het aantal nematoden, de voedselbeschikbaarheid en de zuurgraad van de bodem gemanipuleerd in veldjes van 1 m^2 door middel van het toedienen van respectievelijk nematicide, kunstmest en kalk. Bovendien werd onderzocht wat de effecten van deze behandelingen waren op de groei van vier veel voorkomende grassoorten in deze velden. Deze grassen waren voorgekweekt in de kas en na drie weken met pot en al ingegraven in het veld. De nematicidebehandeling bleek geen significant effect te hebben op de productiviteit van de vegetatie, ondanks een reductie van het aantal nematoden met 70-85%. De enige biomassatoename (+72%) na nematicidebehandeling werd gevonden in bemeste veldjes van een laagproductief grasland. In dit veld was het aantal nematoden na bemesting zeer sterk gestegen, wat klaarblijkelijk heeft geleid tot een aanzienlijke nematodenschade. Dit was tegengesteld aan de verwachting dat bemesting de schade door nematoden juist zou kunnen beperken, doordat een groter wortelstelsel ten gevolge van bemesting leidt tot een lagere nematodenaantasting per gram wortel. Hieruit blijkt dat de gevoeligheid van planten voor nematodenaantasting niet perse hoger is bij een lage voedselbeschikbaarheid. Bovendien nam de wortelbiomassa van de late successiesoort *F. rubra* in een laat successiestadium toe na nematicidebehandeling (+67%).

Dit zou kunnen duiden op de aanwezigheid van soortspecifieke groeireductie door nematoden.

In alle drie de experimenten (hoofdstuk 5, 6 en 7) werd het aantal plantenparasitaire nematoden sterk beïnvloed door de verschillende behandelingen. In de kasexperimenten en in de ingegraven potten in het veld nam het aantal plantenparasitaire nematoden sterk af na bemesting. Dit is waarschijnlijk het gevolg van een direct toxisch effect van de toegediende voedingsoplossing op de nematoden. Ook in de behandelde veldjes nam het aantal nematoden kort na bemesting af, maar nam het aantal op de langere termijn echter toe. Bekalken had over het algemeen een negatief effect op het aantal nematoden. Aangezien de N concentraties in de wortels toenam na bemesting en afnam na bekalken, lijkt het er op dat het aantal nematoden sterk bepaald wordt door de N concentraties in de wortels. Deze resultaten ondersteunen de eerder genoemde conclusie dat kwalitatieve veranderingen in de vegetatie de successie van plantenparasitaire nematoden bepalen.

Het aantal plantenparasitaire nematoden verschilde ook sterk tussen de verschillende plantensoorten. In alle drie de experimenten werd een hoger aantal nematoden gevonden onder vroege dan onder late successiesoorten (hoofdstuk 5, 6 en 7). In een vierde experiment is de specificiteit onderzocht van zes verschillende nematodensoorten voor verschillende plantensoorten (hoofdstuk 8). Hierin werd echter geen relatie gevonden tussen de reproductiesnelheid van de zes nematodensoorten onder zes plantensoorten uit verschillende successiestadia en de habitatvoorkeur van de gastheer (waardplant). Bovendien heb ik geen relatie gevonden tussen de reproductiesnelheid van de nematodensoorten onder de verschillende plantensoorten en het gezamenlijk voorkomen van de nematoden- en plantensoorten in het veld. Dat wil zeggen dat een nematodensoort niet per definitie meer voorkwam in een veld dat gedomineerd werd door een plantensoort waarop deze nematodensoort zich goed kon reproduceren. Klaarblijkelijk hangt de ruimtelijke verdeling van plantenparasitaire nematoden niet alleen af van de aanwezigheid van goede waardplanten, maar hangt dit voor een groot gedeelte ook af van andere factoren zoals interspecifieke concurrentie tussen nematodensoorten en abiotische omstandigheden.

Schattingen van de consumptie door nematoden (hoofdstuk 4) en hun effect op de concurrentie tussen plantensoorten (hoofdstuk 6) laten zien dat plantenparasitaire nematoden een potentieel belangrijke biotische factor zijn die de vegetatiesuccessie kan bepalen. De gevonden afname in aantal en biomassa van plantenparasitaire nematoden bij een lagere voedselbeschikbaarheid heeft echter ook als consequentie dat de nematoden een lagere bijdrage leveren aan de voedselkringloop in de bodem en een geringere hoeveelheid

plantenmateriaal consumeren (hoofdstuk 4). Als gevolg daarvan kan de nematodenschade aan planten bij zowel hoge als lage voedselbeschikbaarheid laag blijven. Dit wordt ondersteund door het feit dat zowel in voedselrijke als voedselarme graslanden, de toediening van nematicide niet heeft geleid tot een hogere biomassa-opbrengst van de vegetatie (hoofdstuk 7). Er is dan ook aanvullend onderzoek nodig om er achter te komen of plantenparasitaire nematoden de vegetatie-successie in de onderzochte graslanden daadwerkelijk beïnvloeden. De huidige resultaten suggereren echter dat de successie van plantenparasitaire nematoden meer bepaald wordt door veranderingen in de vegetatie dan de vegetatiesuccessie door nematoden.

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Curriculum Vitae

Bart Christiaan Verschoor werd op 21 februari 1968 geboren te Vlaardingen. Hij behaalde in 1986 het VWO-diploma aan het Niftarlake College te Maarssenbroek. In september 1986 begon hij aan de studie Plantenziektekunde aan de Landbouwuniversiteit Wageningen. Tijdens deze studie kwam al snel zijn interesse boven drijven voor de ecologie van plant en dier, maar in het bijzonder voor een groep dieren die maar al te vaak over het hoofd wordt gezien, de nematoden. Deze interesse uitte zich in een afstudeervak bij de vakgroep Nematologie waar hij onderzoek heeft gedaan aan de successie van nematoden in het Hulshorsterzand, en een stageperiode bij het Institute for Grassland and Environmental Research in Hurley, Engeland, waar hij onderzoek gedaan heeft aan plantenparasitaire nematoden in graslanden. Daarna heeft hij tijdens een afstudeervak Bosecologie bij het Biologisch Station te Wijster gewerkt aan de ecologie van saprotrofe schimmels van het geslacht *Clitocybe* en *Collybia*.

In 1992 studeerde hij af en begon in februari 1993, ter vervanging van zijn militaire diensplicht, te werken bij het NIOO-CEMO in Yerseke. Gedurende deze diensttijd en een korte aanstellingsperiode als toegevoegd onderzoeker heeft hij meegewerkt aan de afronding van een tienjarig onderzoek aan de successie van loopkevergemeenschappen na indijking van het Markiezaatmeer. In december 1994 is hij aangesteld als assistent in opleiding bij het Biologisch Station in Wijster van de Landbouwuniversiteit Wageningen. Hier heeft hij onderzoek gedaan aan de interacties tussen planten en plantenparasitaire nematoden in verschillende stadia van verschralingsuccessie in graslanden. Dit onderzoek, waarvan de belangrijkste resultaten zijn beschreven in dit proefschrift, heeft hij in 2001 afgerond. Tussen het afronden van zijn proefschrift door heeft hij in 2001 als tijdelijk onderzoeksmedewerker bij de leerstoelgroep Bodembiologie en Bodemkwaliteit meegewerkt aan een onderzoeksprojekt over de relatie tussen boven- en ondergrondse biodiversiteit en het functioneren van ecosystemen.