Terrestrial nematodes in a changing environment

Promotor: dr. ir. A.F. van der Wal
hoogleraar in de nematologie

Co-promotor: dr. ir. A.M.T. Bongers
universitair docent
vakgroep Nematologie

R.G.M. de Goede

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Proefschrift

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
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Agricultural University
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STELLINGEN

1. C-P driehoeken zijn een goed instrument om veranderingen in het milieu vast te stellen.

   Dit proefschrift

2. De nematodenfauna van onze bossen op zure hogere zandgronden vertoont verschijnselen van degeneratie.

   Dit proefschrift

3. De trend dat lichaamsafmetingen van dieren toenemen bij voortschrijdende successie van hun habitat wordt niet gevolgd door terrestrische nematoden.

   Odum, E.P. (1969) Science 164; 262-270.

   Dit proefschrift

4. Bij de bestudering van de oecologische funktie van terrestrische nematoden in een bodemprofiel is een onderverdeling van het bodemprofiel in centimeters niet zinvol indien deze niet kan worden gerelateerd aan een bodemkundige indeling in horizonten.

5. De ontwikkelingen op het gebied van de biologische bodembeoordeling met nematoden vereisen een uitbreiding van middelen voor autecologisch en α-taxonomisch onderzoek aan vrijlevende nematoden.

   Ferris, V.R. & J.M. Ferris (1989) J. Nematology 21: 308-314.

   Bernard, E.C. (1992) Biol. Fertil. Soils 14: 99-103.

   Yeates, G.W., T. Bongers, R.G.M. de Goede, D.W. Freckman & S.S. Georgieva (in press) J. Nematology.

6. Paratypes dienen gedistribueerd te worden over verschillende en toegankelijke collecties.

7. C.O. Nielsens publicatie "Studies on the microfauna II. The soil inhabiting nematodes" zou verplichte kost moeten zijn voor de ecologisch nematoloog van de 20ste eeuw.

C.O. Nielsen (1949) Natura Jutlandica 2: 1-131.

8. Multi-trophic-level interactions are not the exception but the norm in any ecological system.

Moore, J.C., H.W. Hunt & E.T. Elliott (1991). In: Barbosa, P, V.A. Krischik & C.G. Jones (eds) Microbial mediation of plant-herbivore interactions, John Wiley & Sons, New York: 105-140.

9. Exploitatie van fossiele brandstoffen is een zegen voor de mens, maar is een ramp voor het milieu.

10. De volgens de wereldleiders "rustgevende" invloed van de historische SALT I en II accoorden, wordt beangstigend overschaduwd door een juist toenemende bezorgdheid over het voortbestaan van een vredige samenleving in Europa.

11. Met de succesvolle herontdekking van de fiets-met-hulpmotor (snorfiets) staat niets een algeheel verbod op het gebruik van de levensgevaarlijke en geluidsoverlast veroorzakende bromfiets meer in de weg.

12. Vluchtgevaarlijke gedetineerden zijn een blok aan het been van onze samenleving en zouden derhalve weer moeten worden veroordeeld tot de kogel met ketting.

Morris & R. Goscinny (1970) De Daltons op vrije voeten. J. Dupuis NV, Sittard.

Stellingen behorend bij het proefschrift van Ron de Goede: *Terrestrial nematodes in a changing environment.*

Wageningen, 1 juni 1993.

Ter nagedachtenis aan A.L.J. van IJzendoorn

Nematodes do not furnish hides, horns, tallow, or wool. They are not fit for food, nor do they produce anything fit to eat; neither do they sing or amuse us in any way; nor are they ornamental (...). Lacking in all these respects, they fail even in furnishing any moral or praiseworthy example; they are not known to be industrious like the ant, or provident like the bee (...), or anything else that is admirable. What claim, then, one may ask, can such beings lay to our attention? (Cobb, 1915)

CONTENTS

VOORWOORD

Lezers die gewend zijn om een boek van voor tot achter te lezen zullen hun nieuwsgierigheid voortkomend uit het citaat van Cobb nog even moeten onderdrukken, aangezien allereerst een paar woorden van welgemeende dank passen.

Tom Bongers was en is nog steeds de grote drijfveer achter het milieuonderzoek met nematoden. Mede dankzij zijn hartstochtelijke promotie van de mogelijkheden die nematoden bieden in dit type onderzoek en het toegankelijk maken van de Nederlandse nematodenfauna, is het oecologisch onderzoek aan nematoden in een stroomversnelling gekomen. Zijn enthousiasme en optimisme hebben mij zeer gestimuleerd. Toos bedankt voor alle gastvrijheid, in de aanvangsperiode in het Wageningse, maar ook nu ik vanuit Drenthe bij jullie aanklop.

Bij de praktische uitvoering van mijn onderzoek ben ik dank verschuldigd aan allen die mij hebben geholpen met het verzamelen van de vele grondmonsters in alle uithoeken van ons landje. In het bijzonder geldt dit voor Hanny van Megen. Ze toonde haar stuurmanskunst op onbegaanbare bospaadjes, verzamelde nauwgezet de 50 x hoeveel grondmonstertjes, was behulpzaam bij de extractie en het tellen van de nematoden en zorgde voor de zeer prettige werksfeer op de afdeling. René Manger heeft me uitstekend op weg geholpen met het identificeren van nematoden. Kwamen wij er samen niet uit en kon ook Tom geen uitsluitsel geven, dan konden wij altijd een beroep doen op de kennis van P.A.A. Loof. S. van der Werf was behulpzaam bij de selectie van onderzoekslokaties en de interpretatie van mijn vegetatieopnamen. Tenslotte wil ik Hester Dekker, Jan Willem Kamerman, Bart Verschoor en Slavka Georgieva bedanken voor de essentiële bijdrage die zij als doctoraalstudent hebben geleverd aan een drietal hoofdstukken van dit proefschrift.

Of great importance to the realization of the thesis were the stimulating discussions with Gregor Yeates. Although busy at the other side of the world, you were lucky to visit the Netherlands every other year. Your valuable comments on the manuscrips always gave me a new heart to try to reach the finishing line. Het uiteindelijk bereiken van die eindstreep is ook mede een verdienste van mijn promotor prof. A.F. van der Wal, die de afronding kritisch heeft begeleid. Eveneens gaat mijn waardering uit naar mijn collega's van het Biologisch Station Wijster, voor de ruimte die ik kreeg voor de afronding van mijn proefschrift.

Satu, Annika en Kaarina wat zou ik hebben moeten beginnen zonder jullie? Even weg uit die wereld van kronkelende aaltjes en protesterende computers. Kiitokset teille positiivisesta suhtautumisestanne ja ajatusteni ohjaamisesta muihin asioihin. Haluan kiittää myös sukulaisiani Suomessa tuesta, jota olen saanut osakseni viime vuosina.

Ron
6 januari 1993.

SAMENVATTING

Nematoden of aaltjes zijn microscopisch kleine ongelede wormen die in zeer grote aantallen in vrijwel iedere bodem voorkomen. Zij leven in de kleine ruimten, poriën, tussen gronddeeltjes en komen voor in zowel droge als waterbodems. Hun aantallen variëren van 8.000-50.000.000 per m^2 en zij zijn daarmee de meest algemene groep van meercellige dieren in de bodem. Hun voorkomen beperkt zich evenwel niet alleen tot bodems, ze worden ook aangetroffen in en op planten en dieren, vaak als parasieten. Ook hun soortenrijkdom is groot. Van Nederland zijn ongeveer 600 soorten beschreven en in een bodemmonster kunnen gemiddeld 30 tot 60 soorten worden aangetroffen. In bodems levende nematoden voeden zich o.a. met bacteriën, schimmels, plantewortels, algen of bodemdieren. Sommige soorten hebben een beperkt, andere een zeer gevariëerd menu. Nematoden vormen een belangrijke schakel in voedselkringlopen in de bodem. En veranderingen in deze voedselkringlopen manifesteren zich in de samenstelling van de nematodenfauna.

De toenemende zorg over de gevolgen van bodemverontreiniging voor de in en op de bodem levende organismen, heeft er onder andere toe geleid dat onderzoek is begonnen naar de mogelijkheid om effecten van bodemverontreiniging en -verstoring aan te tonen en om ontwikkelingen die onder andere het gevolg zouden kunnen zijn van overheidsbeleid te kunnen voorspellen en controleren. Het onderzoek dat in dit proefschrift is vastgelegd, maakte deel uit van een project waarin wordt onderzocht of verontreinigingen en verstoringen kunnen worden bepaald aan veranderingen in de nematodenfauna. Er diende antwoord gegeven te worden op de volgende vragen:

1. Kan de studie van de nematodenfauna bijdragen aan de vaststelling van de kwaliteit van het bodemleven?
2. Bieden gegevens over de nematodenfauna perspectieven bij de ontwikkeling van een ecologische bodemtypologie die als referentie kan dienen bij de beoordeling van de kwaliteit van bodems?

Een ecologische bodemtypologie omvat niet alleen een beschrijving van bodemleven en vegetatie, maar ook een beschrijving van de chemisch-fysische samenstelling van de bodem, zoals grondsoort en zuurgraad.

Naast een gevoeligheid voor bepaalde pesticiden is in de laatste decennia ook gebleken dat nematoden gevoelig zijn voor verstoringen van verschillende aard, zoals verontreinigingen met zware metalen en andere toxische stoffen, bemesting en verzuring. Om de kwaliteit van het bodemleven aan de hand van de nematodenfauna te kunnen beoordelen moet een eenvoudig te hanteren, doeltreffend meetinstrument beschikbaar zijn. In hoofdstuk 2 is aangetoond dat een beoordelingsmethode gebaseerd op de verhoudingen tussen aantallen nematoden met verschillende leefwijze, perspectieven biedt bij de signalering van veranderingen in het bodemleven. De nematoden kunnen naar hun levenswijze worden ingedeeld in een aantal groepen, met aan de ene kant "kolonisten" (colonizers), nematoden die snel (dagen) in aantal kunnen reageren op veranderingen in het voedselaanbod, en aan

de andere kant "persisters", nematoden met een relatief lange levenscyclus die slechts langzaam reageren op een verhoogd voedselaanbod. In hoofdstuk 2 werd een grafische methode beschreven waarbij de totale nematodenfauna wordt weergegeven met behulp van drie klassen: de *c*olonizer-*p*ersister (c-p) driehoek. Met deze driehoek kunnen veranderingen in de structuur van de nematodenfauna worden beschreven die herleid kunnen worden tot bepaalde typen verstoringen, zoals die welke gepaard gaan met een toename van de beschikbaarheid aan makkelijk afbreekbaar organisch materiaal (zie pijl 1 in Fig. 2.4) of met een toxische werking (zie pijl 2 in Fig. 2.4).

In hoofdstuk 3 wordt de bruikbaarheid van de c-p driehoek onderzocht in een experiment waarin werd getracht de effecten van verzuring en een verhoogde stikstofdepositie op de groei van grove-den door bekalking en bemesting te bestrijden. Bekalking van het bos was in beide onderzoeksjaren, respectievelijk drie en vier jaren na de toediening van de kalk, nog duidelijk gerelateerd aan het voorkomen van bepaalde soorten nematoden. Analyse van de patronen in de c-p driehoeken gebaseerd op de eigen onderzoeksresultaten en op die van een reeks van soortgelijke onderzoeken uitgevoerd door andere instituten op lokaties met verschillende nematodenfauna's, toonde aan dat bekalking resulteerde in een zelfde type verschuiving in de nematodensamenstelling: een toename van het aandeel kolonisten. Dit effect leek pas op te treden na verloop van een aantal maanden maar kon ook jaren later nog steeds worden vastgesteld. Toediening van ureum daarentegen, resulteerde slechts in een kortstondige acute toename van het aantal kolonisten.

Onder experimentele omstandigheden zoals beschreven in hoofdstuk 3, kon de reactie van de nematodenfauna op een te onderzoeken behandeling worden vergeleken met de samenstelling van de nematodenfauna in de ongestoorde "controle" situatie. Onder niet-experimentele omstandigheden ontbreekt echter veelal een "controle" situatie. In dergelijke situaties kan de nematodenfauna van te beoordelen bodems worden vergeleken met die van referentiebodems (ecologische bodemtypologie), of kan de nematodenfauna op opeenvolgende tijdstippen worden onderzocht (monitoring) en kunnen optredende veranderingen in de samenstelling van de nematodenfauna worden geïnterpreteerd.

De ontwikkeling van een systeem van referentiebodems vereist kennis van het voorkomen van nematoden in relatie met omgevingsfactoren, zoals vegetatie en chemisch-fysische bodemparameters. Ten behoeve van onderzoek naar deze relaties werd een inventarisatie uitgevoerd van de nematodenfauna in meer dan 200 grondmonsters die werden verzameld in uiteenlopende vegetatietypen op een verscheidenheid aan bodemtypen (hoofdstuk 4).

De resultaten toonden aan dat het voorkomen van nematoden in belangrijke mate bepaald werd door eigenschappen van hun leefomgeving. Vooral de grondsoort bleek van invloed te zijn op de soortensamenstelling. Daarnaast speelde ook de vegetatie een rol, waarbij in eerste

instantie onderscheid gemaakt kon worden tussen nematodengemeenschappen van bossen, van struwelen en open plekken in bossen, en van graslanden. Er werden met behulp van multivariate analyse technieken (clustering, correspondentie analyse) zeven groepen (Sample Groups) nematodengemeenschappen onderscheiden, die tevens bodem- en vegetatiekundig konden worden getypeerd. Bij de indeling in Sample Groups bleken invloeden van methodische aspecten zoals bemonsteringstijdstip en -strategie van ondergeschikt belang. Geconcludeerd kon worden dat de nematodenfauna deel kan uitmaken van een referentiesysteem in de vorm van bijvoorbeeld een ecologische bodemtypologie.

Beoordeling van veranderingen in de samenstelling van de nematodenfauna in monitoringonderzoek vereist inzicht in de ontwikkelingen in de samenstelling van de nematodenfauna onder invloed van natuurlijke veranderingen in hun leefomgeving. Daartoe werd een beschrijving gemaakt van de ontwikkelingen binnen de nematodenfauna gedurende natuurlijke successie van de vegetatie in een stuifzandgebied (hoofdstukken 5 en 6). De onderzochte successiestadia waren achtereenvolgens onbegroeid stuifzand, een Heidespurrie-Buntgras vegetatie, vijf stadia grove-dennenbos variërend in leeftijd van 3 tot 105 jaar en een overgangsvariant van grove-dennenbos naar Berken-Eikenbos.

De samenstelling van de nematodengemeenschap bleek afhankelijk van het successiestadium van de vegetatie, het vegetatietype (stuifzand, onbebost, bos) en de bodemlaag en werd in belangrijke mate bepaald door onder meer microklimatologische factoren, bodemstructuur en de beschikbaarheid van voedsel. Bij de overgang van onbegroeid stuifzand naar de Heidespurrie-Buntgras vegetatie en vervolgens naar het grove-dennenbos traden relatief grote verschuivingen op in de samenstelling van de nematodenfauna, waarbij soorten verdwenen en andere verschenen. De ontwikkeling van de nematodenfauna van de beboste stadia daarentegen verliep meer geleidelijk.

Met de aanwezigheid van grove-dennen ontwikkelde zich tevens een strooisellaag van naalden, takken etc.. Afhankelijk van de leeftijd van het grove-dennenbos kunnen binnen de strooisellaag op grond van het afbraakstadium van de dennenaalden verschillende sub-lagen (horizonten) worden onderscheiden. Analyse van de nematodenfauna van de bodemhorizonten in de beboste successiestadia toonde aan dat de verschillen in de samenstelling van de nematodenfauna tussen de diverse horizonten binnen één successiestadium groter waren dan de verschillen tussen de successiestadia van één bodemhorizont. Binnen iedere bodemhorizont konden met behulp van de c-p driehoek, patronen in de ontwikkeling van de nematodenfauna worden vastgesteld. Deze patronen bleken gerelateerd aan het successiestadium van de vegetatie en waren afhankelijk van de bodemhorizont. In de diepere bodemhorizonten (fermentatie- en humushorizont van de strooisellaag en in de onderliggende 0-10 cm minerale bodem) volgde de nematodenfauna een ontwikkeling die aangegeven is met pijl 4 in Fig. 2.4: initiële successiestadia met een nematodenfauna voornamelijk bestaand uit taxa behorend tot

c-p groep 2, gevolgd door een toename van het aandeel persisters (c-p groep 3-5) in de loop van de successie. In de bovenste (litter) horizont van de strooisellaag daarentegen werd een dergelijke ontwikkeling van persisters niet vastgesteld, maar bestond de nematodenfauna steeds uit colonizers (c-p groepen 1 en 2), waarbij de meest extreme colonizers (c-p groep 1) in relatief hoge aantallen voor konden komen.

Tenslotte werden de ontwikkelingen in de samenstelling van de nematodenfauna gedurende de natuurlijke successie van de vegetatie in het stuifzandgebied vergeleken met de "Sample Group"-indeling uit hoofdstuk 4 (Fig. 5.7). De op de samenstelling van de nematodenfauna gebaseerde classificatie van de verschillende successiestadia in de "Sample Group"-indeling, bleek in overeenstemming met de bodem- en vegetatiekundige typering van de Sample Groups. Tevens werd aangetoond dat de samenstelling van de nematodenfauna zich als gevolg van natuurlijke veranderingen in hun leefomgeving zodanig kan wijzigen dat klasse- of typegrenzen binnen een (ecologische) bodemtypologie kunnen worden overschreden.

SUMMARY

Increasing awareness of the nature and extent of soil pollution on soil biota and their role in soil processes has resulted in exploring the possibilities of biological assessment systems to indicate the ecological condition of soils and to predict the ecological efficacy of e.g. policy measures. The research presented in this thesis is part of a project in which the possibilities of nematodes as bioindicator organisms are studied. The objectives of the study were to investigate i. the use of the nematode fauna to assess soil quality, and ii. the prospect of the nematode fauna to contributing to an ecological soil classification which can serve as a reference to assess soil quality. An ecological soil classification is based on a description of soils consisting of a set of both biotic and abiotic parameters.

Besides the effects of certain pesticides on the occurrence of nematodes, many studies within the last decades showed changes in the composition of the nematode fauna due to e.g. heavy metals and other pollutants, fertilization and acidification. To assess soil quality by means of analyzing the composition of the nematode fauna, an effective and reliable instrument is needed. In Chapter 2 an assessment method based on life strategies of nematodes is described. Nematode taxa can be classified from *c*olonisers (r-strategists in the loose sense) to *p*ersisters (K-strategists *sensu lato*) on a c-p scale. This c-p scale was divided into three classes, and of any soil sample the proportion of the nematode fauna belonging to each of these three classes was calculated. C-P triangles were used to visualize the distribution of the three c-p classes within a sample. It was shown that c-p triangles could be used to describe patterns in the composition of the nematode fauna which were found to be related to certain disturbances such as acute toxic compounds (arrow 2 Fig. 2.4) or those resulting in an increased availability of easily decomposable organic compounds (arrow 1 Fig. 2.4).

In Chapter 3, c-p triangles were applied to study changes in the composition of the nematode fauna of a Scots pine forest, resulting from the application of lime, additional nutrients and organic manure to restore nutrient availability for tree growth. Soil samples were taken three and four years after the first applications, and in both years the occurrence of nematodes was significantly effected by liming. Analyses of patterns within the c-p triangles based on our own data and on a selection of literature data originating from locations with different nematode faunae showed that following liming, colonizers increased proportionally. This increase became manifest months later and could be demonstrated several years later. Application of urea also resulted in an increased abundance of colonizers. However, the effects of urea were stronger, appeared within a few weeks of application, but seemed to last for only a few months.

In experimental studies like those described in Chapter 3, control treatments usually are available. In general, however, the assessment of the pollution or disturbance of soils lacks such controls. A reference system (e.g. ecological soil classification) or an analysis of

changes in the composition of the nematode fauna using a time sequence, can then be used to assess the nematode fauna of that soil.

Development of a reference system depends upon, among other things, knowledge of the ecological relationships of nematodes to their biotic and abiotic environment. Relationships between soil characteristics, vegetation and composition of the nematode fauna are described in Chapter 4, where the nematode fauna of >200 soil samples taken from a variety of habitats differing in vegetation (forest, shrubs, heathland, grassland) and soil type (clay, loam, sand) was studied. Using multivariate analysis techniques (clustering, correspondence analyses), the nematode fauna of these sites was classified in "Sample Groups". Seven Sample Groups could be distinguished, and these could also be described by soil characteristics in combination with vegetation. Gradual changes in the composition of the nematode fauna were observed in the sequence sandy, loamy sandy, sandy loam, loam soils. However, the nematode fauna of clay soils differed significantly from the former. In soils with comparable physical and chemical characteristics, subdivision was found to be related to vegetation, as was shown for the sandy soils. In sandy soils both the forests and the forest gaps, shrubs and grasslands had their characteristic nematode fauna. Methodological aspects as date of sampling and sampling strategy did not affect the Sample Group classification. It was concluded that the nematode fauna can contribute to a reference system such as an ecological soil classification.

Assessment of changes in the composition of the nematode fauna in monitoring studies requires knowledge of the development of nematode faunae in natural habitats. Long-term changes in the composition of the nematode fauna were studied in a natural primary vegetation succession of blown-out areas in a drift sand landscape (Chapters 5 and 6). The successional stages studied were bare drift sand, *Spergulo-Corynephoretum*, Scots pine (*Pinus sylvestris* L.) forests of respectively 3-5, 25-30, 45-50, 80-90 and 105 years old and an early variant of *Betulo-Quercetum*. Nematode samples were taken from the 0-10 cm mineral soil, and in the forested stages also from the litter, fermentation and humus horizons.

The occurrence of nematodes depended on the stage of succession, the vegetation type (drift sand, *Spergulo-Corynephoretum*, forest) and the soil horizon, and could be related to microclimatological characteristics, soil texture and food availability. Relatively large differences in the composition of the nematode fauna were observed when going from drift sand to *Spergulo-Corynephoretum*, and subsequently to Scots pine forest. Changes in the composition of the nematode fauna during forest development appeared more gradual.

Analyses of the nematode fauna of the forested sites showed a higher similarity between the nematode faunae of comparable soil horizons than between different horizons within the same profile. Within each soil horizon, c-p triangles showed patterns in nematode fauna

development which could be related to vegetation succession and which differed per horizon. In the 0-10 cm mineral soil and in the fermentation and humnus horizons of the forested soils nematode development followed patterns as indicated by arrow 4 in Fig. 2.4: an initial dominance of taxa belonging to c-p group 2, followed by a relative increase of persisters (c-p group 3-5). However, in the litter horizons such development of persisters could not be observed. In all successional stages of the litter horizon, the nematode fauna was composed mainly of colonizers (c-p groups 1 and 2), and here the most extreme colonizers (c-p group 1), could be found in relative high numbers.

Finally, the nematode fauna in the natural vegetation succession of the blown-out areas in the drift sand landscape was compared with the Sample Group classification of Chapter 4 (Fig. 5.7). Projection of the nematode samples taken from the vegetation succession onto the correspondence analysis graph of the Sample Group data, showed that the position of the successional samples coincided with the Sample Groups composed of samples with related habitat types. These similarities between nematode fauna structure and habitat type as found in both investigations, support a classification of soils based on the composition of the nematode fauna as presented in Chapter 4. Moreover, with reference to an ecological soil classification, this comparison showed that developmental changes of the nematode fauna as a result of natural environmental changes in habitat structure can cross boundaries between "classes" or "types".

GENERAL INTRODUCTION

SOIL CLASSIFICATION AND SOIL ASSESSMENT

Soil quality and human life are inevitably connected. Soil offers a place to live on, and we depend largely on soil for our food supply. Moreover, similar relationships apply to many other organisms, whether they live emerged or submerged in the soil. As our soils are exposed to increasing levels of pollution, ecological processes are threatened. In the Soil Protection Act (1986), the Dutch government established high standards for the protection and recovery of, among others, the "ecological function" of soils. The ecological function of a soil can be defined as the suitability of that soil for the organisms living in or on it (Gleichman-Verheijen *et al.* 1991).

To address questions concerning the maintenance or recovery of the ecological functioning of soils, ecological assessment of these soils is needed. Therefore, knowledge of the ecological functioning of soils is essential. Moreover, to identify disturbances and/or pollution and to predict the consequences of events or effects of policy measures on ecosystems, an ecological soil classification should be established to serve as a reference (Gleichman-Verheijen *et al.* 1991, Klijn *et al.* 1991). Such ecological soil classification and ecological soil assessment systems will by definition be based on both abiotic and biotic parameters. In addition to chemical and physical analyses, consideration of biotic parameters provides information of a. "biological availability" (including fluctuations in time) of compounds and their toxicity, b. effects of compounds which were/could not be analyzed chemically, c. past events, d. complex synergistic effects of co-occurring compounds and e. effects of long-term exposure to relatively low concentrations, including bioaccumulation (Zonneveld 1984, Bongers 1990a, Kuyper 1990).

Gleichman-Verheijen *et al.* (1991) presented a scenario to achieve an ecological description of soils. The biotic part of such a description should consist of parameters representing information of the microflora, flora and fauna. Nematodes are among the soil fauna groups which are pre-eminently suitable to be included in an ecological soil classification (Bongers 1990a, Bongers & Van de Haar 1990, Bongers & Schouten 1991) and offer possibilities to assess soil quality (Samoiloff 1987, Freckman 1988, Bongers 1990b, Bongers & Van de Haar 1990).

NEMATODES; GENERAL BIOLOGY

Nematodes are aquatic, bilaterally symmetric, unsegmented, triploblastic, elongated roundworms. They consist of a body wall, digestive, nervous, excretory, and reproductive system, but lack a typical circulatory, respiratory, and perhaps, endocrine system. Their cuticle is a flexible exoskeleton which is supported by internal hydrostatic pressure, and extend inward at all body openings, including the stoma and oesophagus lumen, anus, vulva, excretory pore, and sensory openings (Freckman & Baldwin 1991).

Most terrestrial nematodes are microscopic, but some species are >10mm long. They occur in almost every habitat and are found in high densities. Approximately 80% of all multicellular animals on earth are nematodes (Bongers & Schouten 1991). A classic description of their wide spread occurrence was given by Cobb (1915):

"If all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, valleys, rivers, lakes, and oceans represented by a film of nematodes." "They occur in arid deserts and at the bottoms of lakes and rivers, in the waters of hot springs and in polar seas where the temperature is constantly below the freezing point of fresh water. They were thawed out alive from Antarctic ice in the far south by members of the Shackelton expedition. They occur at enormous depths in Alpine lakes and in the ocean."

Although nematodes are commonly found in terrestrial environments, they are in fact aquatic organisms. Their activity primarily depends on the presence of water films around soil particles. However, a number of nematode species can survive periods of insufficient water availability in a state of anhydrobiosis. Respiration occurs through the body wall and thus, as a consequence of their aquatic life style, depends on the diffusion rates of the respiratory gasses in water. As these diffusion rates are lower than in air, nematodes will often been subjected to low oxygen concentrations.

The Phylum Nematoda is characterized by a high species diversity. Andrassy (1992) estimated that >11000 free-living (i.e. excluding parasites of animals and man) species are described (5450 marine and 5600 continental species). About 600 terrestrial and freshwater nematode species (Bongers 1988) and about 400 marine nematode species (Bongers & Van de Haar 1990) are known to occur in the Netherlands. Usually the species richness of single sites is high, with an average of 30-60 species per soil sample (Bongers 1988).

Probably as a result of the embryological state of the science of nematology (Freckman & Baldwin 1991), the approaches to the classification of nematodes are

subjected to considerable motion. The classification of nematodes species can be based on phylogenetic relationships and on ecological characteristics. Examples of the use of ecological characteristics as a classification of terrestrial nematodes are those based on feeding biology (trophic function) and on life strategies: colonizers versus persisters (Bongers 1990b). In the most recent trophic classification of terrestrial nematodes, seven groups were distinguished (1) plant feeding, (2) hyphal feeding, (3) bacterial feeding, (4) substrate ingestion, (5) predation on animals, (6) unicellular eucaryote feeding, (7) dispersal/infective stages of parasites and (8) omnivores (Yeates *et al.* 1993a). From this it can be seen that nematodes are represented at most trophic levels of the food web. Moreover, they are thought to be closely connected to, and to reflect, fundamental ecological processes (e.g. decomposition, mineralisation, primary production) in soils (Freckman 1988, Coleman 1986, Ingham *et al.* 1985, Yeates 1979, Yeates & Coleman 1982).

NEMATODES AND BIOINDICATION

As do other biota, nematodes respond to changes in environmental conditions. Since the second half of the 70's, a number of studies on changes in the occurrence of nematodes at species and community level as a result of various experimental environmental stresses have shown their usefulness as indicators of the effects of e.g. heavy metals (Bongers *et al.* 1991, Cantelmo *et al.* 1979, Sturhan 1989, Weiss & Larink 1991), chlorophenols (Cantelmo & Rao 1978, Kappers & Wondergem-van Eijk 1988), acidification (Hyvönen & Persson 1990, Schouten & Van der Brugge 1989, Ruess & Funke 1992), deposition of nitrogenous compounds (Tamis 1986), and application of lime (Hyvönen & Huhta 1989, Hyvönen & Persson 1990, Ratajczak *et al.* 1989) and manure (Ettema & Bongers 1993).

In several experiments, the recovery of the nematode fauna after disturbance has been studied (Kappers 1990, Kappers & Manger 1990, Yeates *et al.* 1991, Ettema & Bongers 1993).

Effects of disturbances act primarily on the individuals present at a site, and thus will, in principle, become visible at the level of specimens or species. However, groups of related nematode species (taxonomical, trophic status, life-history pattern) will often show similar response(s) to certain kinds of stress. Dorylaimids, for example, are imputed to be highly sensitive to various kinds of disturbances such as heavy metals, fertilization, forest grazing, and forest cutting (Zullini & Peretti 1986, Sohlenius & Wasilewska 1984, Hyvönen & Huhta 1989, Johnson *et al.* 1974, Ferris & Ferris 1974, Wasilewska 1979). Species belonging to Rhabditida are found to increase when levels of easily decomposable

organic compounds are elevated (Sohlenius 1973, Sohlenius & Wasilewska 1984). In laboratory studies Schiemer (1983) showed that population development occurred only when certain threshold densities of their food i.e. bacterial cells were attained. Increased densities of field populations of species belonging to Rhabditida are therefore thought to be related to increased bacterial productivity (Sohlenius 1973, Bååth *et al.* 1981).

Based on such nematode groupings, several indices have been developed which are indicative of different kinds of environmental changes. Examples of indices based on trophic function and life-history patterns are fungivore/bacterivore ratio (e.g. Sohlenius & Boström 1984, Freckman & Ettema 1993), trophic structure index T (Freckman & Ettema 1993), proportion of "dauer larvae" within Rhabditida (Sohlenius & Boström 1984) and the Maturity Index and Plant Parasite Index (Bongers 1990b). As these indices are based on ecological characteristics of the nematode species involved, they differ from indices which describe structural aspects of the nematode fauna, e.g. the various diversity and evenness indices. The latter methods, including statistical techniques such as k-dominance curves and species-abundance plots (Heip et al 1988), found application especially in the detection of pollution in marine habitats (Shaw *et al.* 1983, Platt 1985).

Besides relationships between the occurrence of nematodes and the environmental disturbance(s), nematodes possess many practical qualities which make them useful to assess environmental conditions (Freckman 1988, Bongers & Schouten 1991):

- They occur in almost every habitat (terrestrial, aquatic and marine), and in polluted or disturbed areas, they are among the last animals to die;
- They occur with many species per sample or habitat;
- They are present at most trophic levels throughout the food web;
- They have relatively short generation times, allowing them to respond quickly to environmental changes;
- They show specific responses to various types of pollution or disturbances;
- They are in close contact with dissolved compounds in the free soil water;
- Due to their low active dispersal ability, nematodes are indicative of the soil horizon in which they occur;
- The sampling of sites is non-destructive and can be done any time of the year;
- They are easily extracted from soils;
- Storage of samples (for future re-examination) requires little space;
- They show low attractiveness to men.

Moreover, many species are easily cultured or manipulated in the laboratory. Finally, the Dutch nematode fauna is easily accessible and identification requires little time and is relatively cheap.

NEMATODES AND ECOLOGICAL SOIL CLASSIFICATION

Specific relationships between the occurrence of nematodes and their environment were predicted in the beginning of the 20th century by Cobb (1915), who supposed that:

"The location of towns would be a corresponding massing of certain nematodes. Trees would still stand in ghostly rows representing our streets and highways. The location of the various plants and animals would still be decipherable, and, had we sufficient knowledge, in many cases even their species could be determined by an examination of their erstwhile nematode parasites."

Approaching the end of the 20th century, our knowledge on the distribution of nematodes in many environments has been extended. In an extensive review on nematode populations in relation to soil environmental factors, Yeates (1981) concluded that the nematode fauna composition reflects soil (texture, porosity, chemistry), climatic (moisture, temperature), plant (species, productivity) and management factors, as well as interactions among the soil biota. Although causal relationships between the occurrence of nematode species and environmental factors are still poorly known, many correlative relationships have been described. Moreover, several studies have indicated the possibility of classifying habitats into groups of comparable environmental characteristics when using the composition of the nematode fauna as a basis for the classification (Johnson *et al.* 1972, 1973, 1974, Arpin 1979, Arpin & Ponge 1986, Bongers *et al.* 1989, Baujard *et al.* 1979, Scotto La Massese & Boulbria 1980, Yeates 1984).

OUTLINE OF THE THESIS

The objectives of the study are to investigate i. the use of the nematode fauna to assess soil quality, and ii. the prospect of the nematode fauna contributing to an ecological soil classification; this ecological soil classification can serve as a reference in assessing soil quality.

In Chapter 2 a new method, the c-p triangle, is described allowing the study of changes in the composition of the nematode fauna as a result of environmental changes or disturbances. The c-p triangle is a graphical presentation of the c-p (colonizer-persister) frequency distribution of the nematode fauna, and can provide information which can be used in addition to the closely related Maturity Index. Application of c-p triangles is shown in the next chapters, where they are used to analyse changes in the nematode fauna

as a result of experimental disturbances (Chapter 3) and of natural vegetation development (Chapter 5).

Using multivariate analyses techniques, the nematode fauna of a variety of habitats differing in vegetation (grassland, heather, shrubs, coniferous and deciduous forest, outer marshes) and soil type (clay, loam, sand) is analysed and classified into groups with similar nematode fauna composition. The classification obtained is related to physical and chemical soil characteristics and to the vegetation of the sites (Chapter 4). Changes in nematode fauna composition (species distribution, community structure, trophic structure, biomass) in relation to natural habitat development are described in a primary succession of blown-out areas in a drift sand landscape (Chapters 5 and 6). The significance of the results to the classification obtained in Chapter 4 are discussed.

GRAPHICAL PRESENTATION AND INTERPRETATION OF NEMATODE COMMUNITY STRUCTURE: C-P TRIANGLES

SUMMARY

Based on life strategy complexes, ranging from colonizers (c) to persisters (p), nematode taxa can be allocated to one of five groups (c-p groups) placed on a c-p scale. Taxa with high r are classified to c-p 1 and taxa with a low r to c-p 5. The weighted mean of the frequency distribution of the c-p groups was proposed as the Maturity Index (Bongers 1990b), an index with the potential to detect disturbances in terrestrial and freshwater habitats. In this study the significance of studying the frequency distribution itself, is demonstrated. C-P triangles are proposed as a helpful tool, facilitating analyses of patterns in c-p frequency distributions of nematode samples. Applications of the c-p triangle are demonstrated, and patterns of change due to disturbances, or as a result of recovery, are discussed.

INTRODUCTION

The nematode fauna of soils and sediments has characteristics that make it suitable for indication and assessment of pollution and other environmental disturbances (Bongers 1990b, Bongers *et al.* 1991, Freckman 1988). When studying changes in the composition of the fauna for e.g. biomonitoring purposes, lists of taxa are difficult to interpret, except perhaps for specialists in a particular field. Therefore, the information within these lists needs to be modified. Although nematode biomass and abundance are often used to indicate changes and to judge the quality of ecosystems, these parameters often show a peak in an eutrophication or production gradient (Pearson & Rosenberg 1978); thus changes in these parameters may not indicate the direction of the change in an environment. Diversity indices and species dominance curves offer better perspectives but in order to compare different situations the level of taxonomic discrimination also has to be comparable. Moreover, as these indices ignore the ecological requirements of the constituent species, diversity may increase in cases where it would be expected to decrease (Bååth *et al.* 1978, Leffler 1978, Rapport *et al.* 1985).

The nematode Maturity Index (MI) (Bongers 1990b) is a community index that contains ecological information. It is based on separation of nematode taxa in five groups (colonizer-persister or c-p groups) based on life strategy complexes. Basically families with a high r are grouped in c-p group 1 (colonizers), and taxa with a low r in c-p group 5 (persisters). Families with intermediate life strategies are classified in one of the intermediate groups.

Details of the rationale for the separation of families in these groups are given in Bongers (1990b) and Bongers *et al.* (1991), and the values have been successfully applied by Freckman & Ettema (1993) and Yeates *et al.* (1993b).

Pollution or other ecological disturbances lead to a numerical change of colonizers, resulting in a nematode fauna in which colonizers (r-strategists *sensu lato*) are prevalent (Bongers *et al.* 1991). Persisters (K-strategists *sensu lato*), which appear to be much more sensitive to pollutants, will decline. The MI was proposed as the mean of the frequency distribution of the c-p groups or the weighted mean of the individual c-p scores of taxa in a given sample.

While the MI, like every index, reduces information, the information lost may be relevant for interpretation. When using the MI, information concerning the distribution of nematode taxa over the constituent c-p groups is lost. MI-decrease can result from an increase in c-p 1 as a result of increased bacterial production, or can result from a numerical decrease of the higher c-p groups as a result of e.g. pollution or acidification. On the other hand, a fauna with MI=2 might indicate an extreme dominance of c-p 2 caused by a given factor, or a well-balanced distribution over the c-p groups with c-p 1 compensating for the higher groups. Examples of increasing numbers of colonizers and a decrease of persisters due to environmental changes resulting from natural events or anthropogenic disturbances, have been given in several studies (Bongers 1990b, Bongers *et al.* 1991, Ettema & Bongers 1993, Chapter 3, Chapter 5). During recovery of ecosystems the opposite changes in nematode fauna structure are expected to occur.

The c-p frequency distribution of samples can be given in tables, but graphical presentation facilitates the interpretation, especially when large data sets or long time series are involved.

C-P TRIANGLES

Ettema and Bongers (1993) showed that rhabditids belonging to c-p group 1, can be compared to 'enrichment opportunists' (Pearson & Rosenberg 1978): they only reach high dominance in food rich conditions and disappear or might, as far as nematodes are concerned, form dauerlarvae as soon as bacterial production decreases below their threshold level. In contrast to c-p 2, these extreme colonizers are replaced by higher groups during subsequent recovery or succession. However, c-p group 2 as a whole remains dominant, and species within this group appear to be among the last surviving environmental pollution (pers. observ.). The higher c-p groups (c-p 3-5) are often low in numbers (Bongers *et al.* 1989), and are hardly influenced by a sudden increase of bacterial production (Ettema & Bongers 1993). Examination of the composition of the groups c-p 1, c-p 2 and c-p 3-5, will thus give

optimal discrimination regarding direct and indirect effects of environmental disturbances on the nematode fauna.

In analogy of the classification of Grime (1979) for vegetation types distinguishing ruderals, stress tolerators and competitors, the c-p groups 1, 2 and 3-5 can be presented in equilateral triangles. Figures 2.1-2.3 show c-p triangles in which the c-p values of many samples can be compared instantly. In the appendix formulas are given for construction of these triangles in a spreadsheet programme.

APPLICATIONS

In the Figs 2.1-2.3 c-p triangles are applied to short- and long-term monitoring studies, and to a large data set of reference sites, respectively.

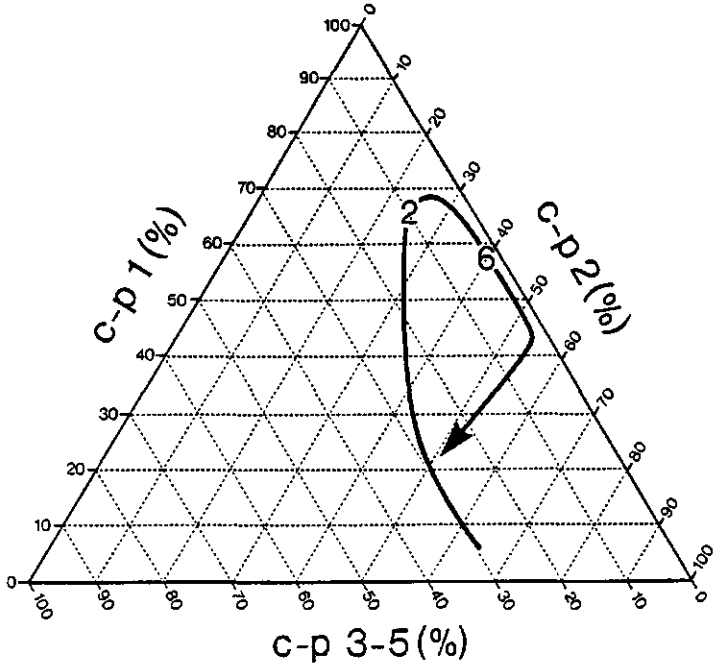

Figure 2.1. C-P triangle of short-term successional changes in c-p value group distribution of the nematode fauna after manuring a fallow soil. Samples were taken at weekly intervals. Temporal changes in nematode fauna structure are given by a drawn line. Week 2 and 6 are indicated by corresponding numbers.

Short-term monitoring

In Fig. 2.1 data are given from a manuring experiment (Ettema & Bongers 1993). Some days after the addition of manure, the proportion of colonizers increased. They reached maximum abundance in week 3, and were subsequently replaced by higher c-p groups. At week 2 (increasing c-p 1 score, following increased bacterial production) and week 6 (decreasing bacterial production), samples were found to have comparable MIs (respectively 1.53 and 1.58) but different c-p value group distributions. The c-p triangle shows that changes in the nematode fauna at disturbance (week 2) and subsequent 'recovery' (week 6) involved changes in the contribution of various c-p groups which were not reflected in the overall MI.

Long-term monitoring

Changes of nematode fauna structure induced by natural succession of the vegetation in a drift sand area are presented in Fig. 2.2. The sequence of successional stages studied was bare drift sand (stage 1), *Spergulo-Corynephoretum* (stage 2), and finally Scots pine forest (stages 3-8) (see Chapter 5 for details). Under the extreme conditions in the bare drift sand, the few nematodes present were predominantly *Eudorylaimus*, an omnivorous genus, well adapted to dehydration, and classified as c-p 4. The c-p value distributions of the successive stages reflect changes in the vegetation. The c-p value distributions showed a typical pattern during the transition from *Spergulo-Corynephoretum* to Scots pine. Colonization of Scots pine in the *Spergulo-Corynephoretum* resulted initially in a relative decrease of c-p 3-5, but was followed by a gradual increase of this group when forest succession proceeded.

Reference sites

Fig. 2.3 shows the c-p value group distribution of the nematode fauna within the 0-10 cm mineral soil of 170 samples taken from 74 locations with more or less natural or slightly managed forest on soil types ranging from sandy loam to slightly loamy sand within the Netherlands. Based on their nematode fauna the samples were classified in five Sample Groups (SG)(Chapter 4) for which contour lines are given in Fig. 2.3. The five SGs could also be described by soil physical and chemical characteristics in combination with vegetation, representing a gradual floristic and pedological sequence with SG C (sandy loam soils with rich hornbeam-beech forests) and SG G (very poor slightly loamy sand soils with Scots pine forests) as the extremes (Chapter 4). As this sequence can be shown in c-p triangles, such triangles may provide a reference and illustrate changes in monitoring studies.

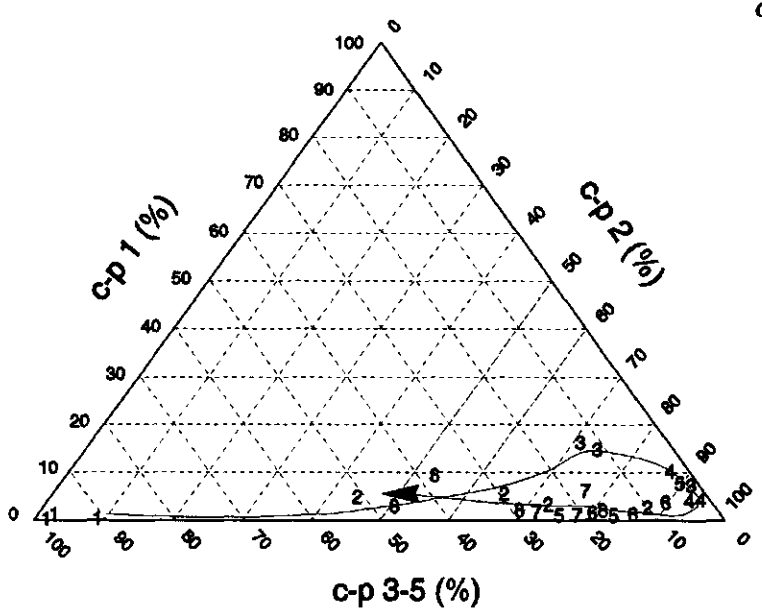

Figure 2.2. C-P triangle of long-term successional changes in nematode fauna composition within the 0-10 cm mineral soil of a primary succession of blown-out areas in a drift sand landscape. Numbers refer to the corresponding stages of succession. Trends are indicated (arrows).

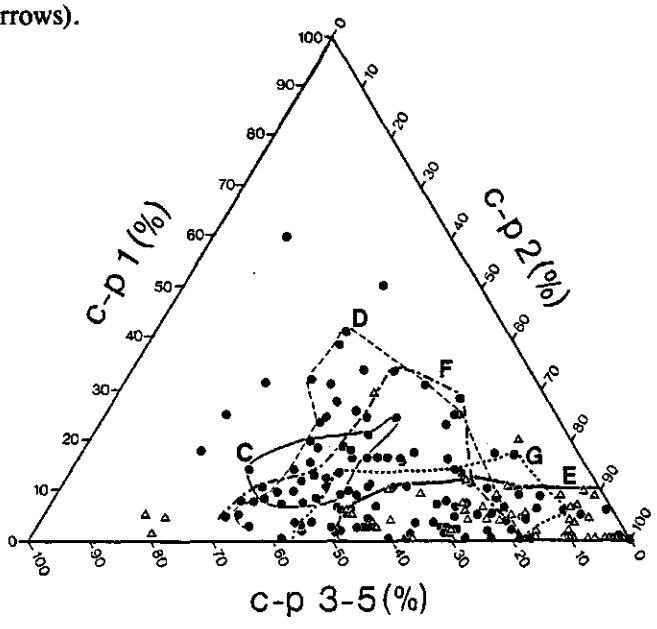

Figure 2.3. C-P triangle of 170 nematode samples taken from 74 locations with more or less natural or slightly managed forest on soil types ranging from sandy loam to slightly loamy sand within the Netherlands. Five Sample Groups (SG C-G) including 90% of the corresponding samples are characterized by contour lines. Samples of SG E are indicated by triangles.

DISCUSSION

One of the features of c-p triangles is that shifts within c-p groups that do not cause shifts in MI can be observed. Under stressed conditions, where bacterial activity is also restrained, the c-p 2 group reaches a high dominance. Abolishing the stresses might result on the one hand in an increased bacterial production and increasing proportion of c-p 1 or, on the other hand, in a recovery of persisters which are no longer hampered. This means that, if colonizers and persisters increase or decrease simultaneously, the MI might remain the same.

For graphical presentation c-p 3, 4 and 5 were combined and therefore information is lost, because a shift from c-p 5 towards c-p 3 does not influence the sample's position. There are indications that separating these groups still remains meaningful. In acid sandy soils, Prismatolaimidae, Teratocephalidae and Diphtherophoridae (c-p 3) are generally present but for the dorylaimids (c-p 5), the environmental conditions appear too extreme (pers. observ.).

The c-p triangle demonstrates relative shifts in the composition of the nematode fauna. Only if the total density of nematode taxa included in the calculation of the MI is given, the shifts can be interpreted in terms of changes in absolute numbers. Although we have tried several possibilities to include the number of nematodes involved in the graphs, we have not yet found a practical method else than indicating absolute abundance by differences in symbol size, or by the substitution of the sample points by numbers corresponding to abundance classes.

The direction of changes can be indicated by arrows (Fig. 2.4). Increase of c-p 1 (arrow 1) indicates increased availability of a bacterial food source (Ettema & Bongers 1993). The relative increase of c-p 1 often will imply an increase in absolute numbers of nematodes as well. This is in contrast to absolute numbers of c-p 2 and/or c-p 3-5, which may not be altered significantly. Although an increase of c-p 1 can be the result of environmental disturbance(s), changes in the composition of the nematode fauna in the direction of arrow 1 do not necessarily have to be interpreted as being negative. In e.g. agricultural systems it could indicate increased fertility (see e.g. Fig. 2.1). Examples of nematode fauna changes following arrow 1 are given in Fig. 2.1 and Chapter 3. Arrow 2 indicates the effects of environmental disturbances (e.g. acidification (Chapter 3), chemical pollution, heavy metals (Weiss & Larink 1991), systematic removal of the organic layer in forests (pers. observ.)) by a relative increase of c-p 2 at the expense of c-p 3-5. The decrease of the latter, will most likely be associated with a decrease in absolute numbers as well. Finally, arrows 3, 4 and 5 indicate recovery or natural succession of a given habitat (see Fig. 2.1 and Fig. 3.4 for recovery, and Fig. 2.2 and Fig. 5.6 for natural succession).

Graphical presentation offers the possibility to define areas within the triangle where disturbance will be unequivocal or can be suspected. The borders of these areas will vary

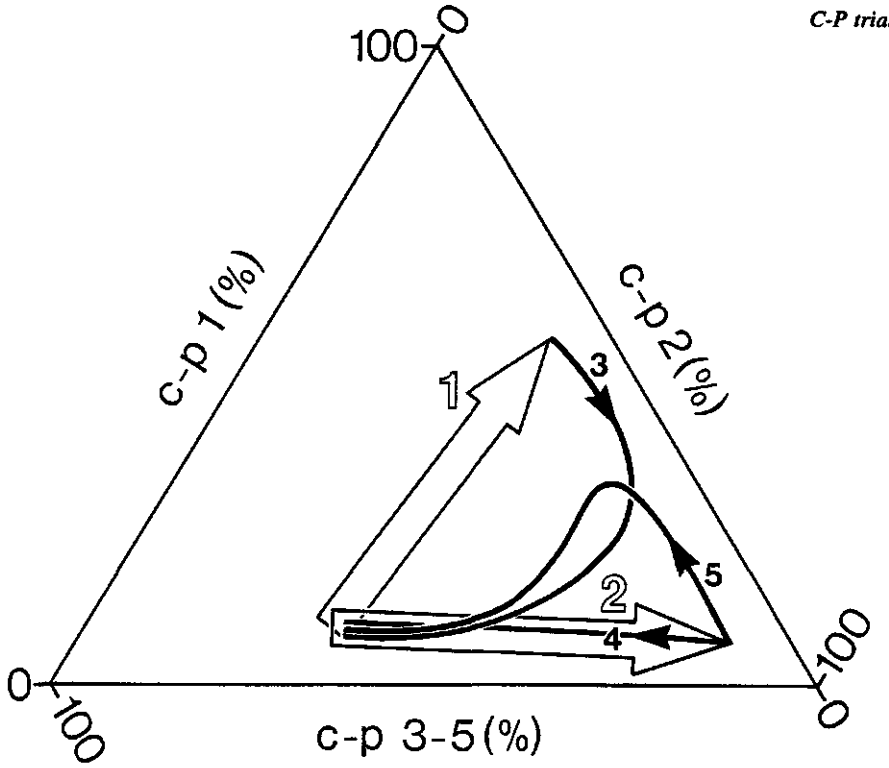

Figure 2.4. C-P triangle schematically showing the main directions along which changes in nematode fauna composition may occur as a result of disturbances (open arrows 1 and 2) and subsequent recovery or natural succession (line arrows 3, 4 and 5). See text for further explanation.

according to the ecological situation and soil type; they have still to be defined.

CONCLUSIONS

When proportions of colonizers and persisters increase or decrease simultaneously, these changes remain unnoticed when using only the MI, but they are detected by using c-p value groups. A graphical presentation of nematode c-p distribution by using c-p triangles, facilitates interpretation of shifts within the c-p groups. These triangles proved also very useful in monitoring studies. They visualize directions of change in nematode fauna structure, indicating e.g. changes in bacterial production, stress conditions or recovery. They can be a tool to assess soil quality.

ACKNOWLEDGEMENTS

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EFFECTS OF LIMING AND FERTILIZATION ON NEMATODE COMMUNITIES IN CONIFEROUS FOREST SOILS

SUMMARY

Effects of fertilization and liming on the nematode community structure of the organic and mineral layer of a Scots pine forest were studied three and four years after first applications. Changes in nematode community structure were found for liming only. In the organic layer, in both years significant changes in the composition of the nematode fauna could be shown, whereas this was found for the mineral layer in the fourth year only. Changes in the composition of the nematode fauna were found only when bulk soil pH had increased. Irrespective to soil layer, Acrobeloides, Protorhabditis *and* Eumonhystera *increased, and* Wilsonema *decreased in the limed plots. C-P triangles proved to be a helpful tool in analyzing effects of forest liming and fertilization at nematode community level. Based on our own data and on a selection of literature data, it was shown that following liming, taxa with low c-p score (r-strategists* sensu lato*) increased in proportion. This increase appeared after months, and could be demonstrated also after several years. Application of urea also resulted in an increased number of taxa with low c-p score. However, the effects were stronger, appeared within a shorter period of time, and seemed to last only for months. Moreover, also the relative number of c-p group 3-5 (K-strategists* sensu lato*) decreased.*

INTRODUCTION

Widespread environmental pollution by atmospherical deposition of a.o. N- and S-compounds, threatens the ecological functioning of soil ecosystems. In the Netherlands average deposition of NO_x, NH_x and SO_x are 1160, 2400 and 670 $mol.ha^{-1}.y^{-1}$ respectively, with local values of more than 1500, 6000 and 1200 $mol.ha^{-1}.y^{-1}$ respectively (Heij & Schneider 1991). Studies are undertaken to monitor changes in plant and animal communities and functional ecosystem parameters in order to reveal the impact of such pollution. On the other hand experiments are started to study possibilities to counteract the deterioration of the soil environment.

The study presented here was part of a multidisciplinary research programme (Harderwijk Forest Fertilization Project) to study whether application of additional nutrients or lime to forest soils, can restore nutrient availability for tree growth, leading ultimately to improved forest vitality (Dilz *et al.* 1990). Although these practices aim to

aid the recovery of the soil ecosystem in favour to plant growth, they will inevitably lead to disturbance of the actual soil biota community. When studying changes induced in the soil biota community structure (e.g. by forest liming and fertilization) therefore, community indices which are sensitive to environmental disturbance are thought to be a useful tool.

To study effects of corrective forest fertilization and liming on soil biota, changes in the nematode community were investigated during two subsequent years. Like other soil fauna, nematode assemblages are thought to reflect the ecological state of the soil environment and because of many supplementary practical and theoretical qualities (e.g. Freckman 1988, Samoiloff 1987) they are considered suitable for monitoring soil quality (Bongers 1990b).

Forest fertilization and liming experiments in Scandinavia and Central Europe (Bassus 1960, 1967, Huhta *et al.* 1986, Hyvönen & Persson 1990, Ratajczak *et al.* 1989) showed effects on total number of nematodes and composition of the nematode community, depending on fertilizer and time of exposure. However, effects on total number of nematodes and trophic structure, proved highly variable. Besides, a comparison of effects at taxa level, is often hampered by differences in species composition between the various experiments.

In this study an attempt was made to generalize the observed changes in nematode community composition by using the Maturity Index (Bongers 1990b) and c-p triangles (Chapter 2). The Maturity Index is a community index based on life strategies of non-plant parasitic nematodes, and the c-p triangles are graphical presentations derived from this Maturity Index. For this purpose, results were obtained from Forest Fertilization Project Harderwijk and from a selection of literature data.

MATERIAL AND METHODS

Site description

The stand, a Scots pine (*Pinus sylvestris*) forest planted in 1960, on a sandy soil, was located in the central part of the Netherlands south east of Harderwijk (52°19'N, 5°40'E). Information on chemical composition of the organic and the mineral layer is given by Dilz *et al.* (1990) and Hekstra *et al.* (1990) for 1988 and 1989 respectively. The forest had very little herb layer, with the *P. sylvestris* providing the only roots for root-feeding nematodes; thus the nematode fauna sampled in the litter and upper 10 cm of mineral soil was primarily that of the decomposer food web.

Our experiment was part of a larger investigation performed to study the influence of mineral and organic amendments on tree-growth in areas with increased nitrogen deposition. The experimental site was divided in 72 plots, each 22x25 m. Three types of treatments were tested separately, viz. the application of the nutrients (K, Mg, P and Ca), liming, and fertilization with organic manure. Each application was carried out in three replicates which were randomized within each treatment.

Based on observed changes in mycoflora (Kuyper & De Vries 1988) and vegetation (Dirkse & Van Dobben 1988), nematode samples were taken from a selection of treatments only. Samples were taken from the K (120 kg K_2O ha^{-1}), Mg (166 kg MgO ha^{-1}), 3 and 9 ton $CaCO_3$ ha^{-1}, and the duck manure (71 kg N ha^{-1}) treatments.

K and Mg were applied in three equal parts in spring 1986, 1987 and 1988. In autumn 1985 all limed plots received 3 ton $CaCO_3$ ha^{-1}. After one year the 9 ton $CaCO_3$ ha^{-1} treatment received the remaining 6 ton $CaCO_3$ ha^{-1}. Duck manure was applied in autumn 1985. The duck manure and lime ($3Ca^+$ and $9Ca^+$) treatments received additional amounts of K, Mg and P (114 kg P_2O_5 ha^{-1}) to reach the same level of fertilization as in the nutrient treatments. Only the 3 ton $CaCO_3$ ha^{-1} treatments were applied also without these additional nutrients, and are indicated as $3Ca^-$.

Sampling, extraction and counting

On 13 and 14 september, 1988 and 20 november, 1989, respectively three and four years after the first applications, the nematode fauna of the six treatments was sampled. In 1988 samples were taken from the three limed treatments ($3Ca^-$, $3Ca^+$ and $9Ca^+$), the duck manure treatment and the control plots. In 1989 the three limed treatments and the Mg and K treatments were sampled including the control plots. All three replicate plots of each treatment were sampled separately.

In the central 12x15 m from each plot twenty cores (core diameter 30 mm) were taken in a regular pattern over the whole area. The cores were split into organic and mineral layers. For each layer the separate cores were combined into one bulk-sample per plot. In 1988 the mineral layer was sampled to a depth of 10 cm, whereas in 1989 only 0-3 cm mineral layer was sampled. In 1988, three bulk samples per plot were collected from the $3Ca^-$ and duck manure treatments to study intra-plot variation.

After each bulk sample was mixed carefully, the nematodes were extracted from 25 g fresh mass litter and 100 g fresh mass mineral soil using a modified Oostenbrink elutriation apparatus (Oostenbrink 1960). The litter was soaked one hour in 400 ml water and cut in a blender (5 sec. in a Braun MX 32) before extraction (Schouten & Arp 1992). Total numbers of nematodes per sample were estimated by counting 10% of the extracted nematode fauna under a low-magnification dissecting microscope. Afterwards the nematodes were heat-killed and fixed in 4% formalin. The generic composition of each

sample was determined under high magnification (400-1000x) in subsamples taken from the bulk suspensions. From every single sample at least 100 individuals were identified to genus according to Bongers (1988). From the three replicate samples taken in the 3Ca⁻ and duck manure plots, the composition of only one organic layer replicate, but all three mineral soil replicates were analyzed.

Water content was determined after 24 h drying at 105°C. pH(KCl) of the mineral and organic layer was determined by using samples of 10 and 5 g fresh mass respectively in 25 ml 1 N KCl.

Statistics

Changes in population structure were analyzed using correspondence analysis and canonical correspondence analysis (CANOCO, Ter Braak 1988). The analyses were based on relative nematode abundance, and the rare genera (occurring in $<0.20\%$ of all samples of a horizon) were excluded from the analyses. Differences in generic abundance between the treatments were tested by Mann-Whitney U-test (Sokal & Rohlf 1981). The taxonomic classification and the classification of the genera into feeding groups followed Bongers (1988) and Bongers *et al.* (1989) respectively. The Maturity Index was calculated according to Bongers (1990b).

RESULTS

Total number of nematodes

In the organic layer, liming tended to reduce the total number of nematodes (Table 3.1). This reduction was significant only after four years. In the mineral soil no significant differences in total number of nematodes were found. Therefore, relative instead of absolute abundances could be adequately used to describe the effects of the treatments on the nematodes.

In 1988 three replicate bulk-samples were collected from each plot of the 3Ca⁻ and duck manure treatments. The mean coefficient of variation for the total number of nematodes within the plots was 17.5 for the organic layer, and 21.2 for the mineral layer.

Community analyses

Organic layer: Fig. 3.1 shows the CCA-plot of the nematode fauna from the organic layer of all plots sampled, with soil pH, treatment and year of sampling as variables. The variation in species composition on the axes is rather low (eigenvalue of first axis is 0.0776) and the first two axes explain 63% of the variance. Despite this low eigenvalue,

Table 3.1. Effect of lime and duck-manure on pH(KCl)[1)] and total number of nematodes[2)] in the organic layer and mineral soil, three and four years after their first applications.

YEAR	ORGANIC LAYER				MINERAL SOIL			
	LIME		MANURE		LIME		MANURE	
	-	+	-	+	-	+	-	+
				pH(KCl)				
1988	3.0 *	5.4	2.9 *	3.2	3.7	3.8	3.4	3.5
1989	2.9 *	5.3	-	-	3.2 *	3.9	-	-
				NEMATODE ABUNDANCE				
1988 mean	3740	2950	3080	2720	1130	1120	770	1000
s.d.	1005	970	355	495	475	265	195	310
1989 mean	4620 *	3380	-	-	1290	1530	-	-
s.d.	1025	515			320	315		

[1)]pH(KCl) mineral soil 1988 and 1989 respectively taken from 0-10 and 0-3 cm; [2)] for organic layer and mineral soil respectively per 25 g fwt and 100 g fwt; *=$p<0.05$; - not determined.

the species-environment correlation of the first axis is 0.925 and is defined by pH ($r=-0.840$, $P<0.01$). A Monte Carlo permutation test showed that the pH significantly explained the variation along the first axis ($P\leq0.01$). The second axis correlates with the year of sampling ($r=0.767$, $P<0.01$).

Three and four years after the beginning of fertilization obvious differences in the structure of the nematode fauna of the organic layer were demonstrated between the plots with and without $CaCO_3$ (Fig. 3.1). No differences in nematode fauna were found between the $3Ca^+$, $3Ca^-$ and $9Ca^+$ treatments. However, there was a significant year effect. The canonical correspondence analyses did not show differences between duck manure, Mg and K and their controls.

In both years, liming resulted in a significant increase in relative abundance of *Acrobeloides* and *Protorhabditis*, a tendency to increase for *Eumonhystera* and a significant reduction of *Wilsonema* (Fig. 3.1, Table 3.2). A significantly lower relative abundance of *Ditylenchus* and *Metateratocephalus* was found three years after Ca-

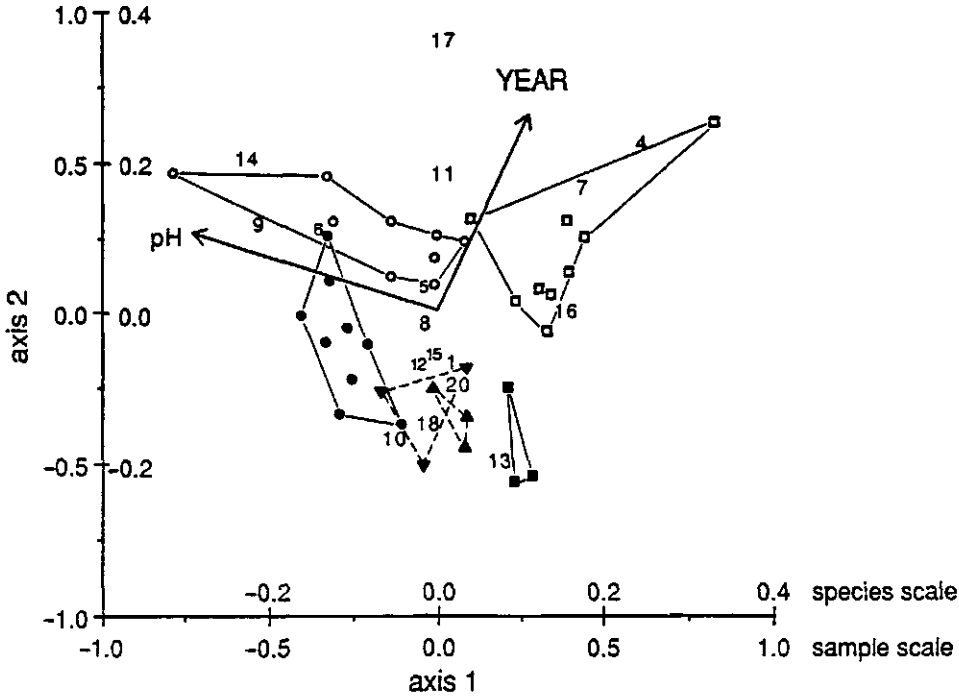

Figure 3.1. Ordination (CCA) diagram for axes 1 and 2 based on the nematode fauna of the **organic** layer, with sites (-lime, ■/□; +lime, ●/○; -duck manure, ▲; +duck manure, ▼; 1988, filled symbols; 1989, open symbols) and taxa (represented by numbers which correspond to table 3.4) indicated. The main environmental variables are represented by arrows. Note that the axes for sites and taxa have differing scales.

application, whereas *Bunonema* had a significantly lower relative abundance after four years only (Table 3.2).

In the organic layer, no significant effect of application of duck manure on any nematode taxon was found (Table 3.3).

Mineral layer: As compared with the organic layer, the nematode community structure in the mineral layer showed less effects of the treatments (Fig. 3.2). The first CCA axis had an eigenvalue of 0.138 and correlated best with the year of sampling ($r=0.861$, $P<0.01$). The second axis (eigenvalue 0.063) had a species-environment correlation of only 0.836. This axis correlated best with pH ($r=-0.589$, $P<0.01$) and application of duck manure ($r=0.576$, $P<0.01$). Thus, most of the variation in the composition of the nematode fauna was correlated with the year of sampling. In the plots without $CaCO_3$ application, *Metateratocephalus*, *Tylenchorhynchus*, *Drilocephalobus*, *Cervidellus* and *Filenchus* had lowest frequency and/or relative abundance after four years (Fig. 3.2,

Table 3.2. Effect of lime on the relative numbers (mean %) and frequency (f[1)]) of the dominant nematode taxa in the organic layer, three and four years after first fertilization of treatments.

GENUS	3 YEARS						4 YEARS					
	- LIME			+ LIME			- LIME			+ LIME		
	mean	s.d.	f	mean	s.d.	f	mean	s.d.	f	mean	s.d.	f
Filenchus	15.0	3.12	3	10.0	5.04	9	8.7	4.04	9	6.6	4.83	9
Ditylenchus	3.4	1.90	3	* 0.9	1.63	3	9.8	7.15	9	5.5	3.66	9
Aphelenchoides	14.6	4.96	3	20.1	5.87	9	21.3	3.97	9	22.7	7.95	9
Protorhabditis	0.6	1.07	1	* 6.8	4.07	5	3.2	2.19	8	*** 8.4	1.66	9
Bunonema	0.0	0.00	0	1.5	2.19	5	6.1	2.20	9	** 3.2	1.65	9
Heterocephalobus	0.6	0.55	2	2.1	2.20	6	2.6	4.11	6	3.7	3.13	9
Acrobeloides	1.9	1.59	3	* 7.5	3.18	9	2.5	1.86	8	** 10.2	5.52	9
Cervidellus	2.5	1.91	3	1.8	2.12	5	0.7	0.81	5	1.6	1.89	6
Drilocephalobus	0.0	0.00	0	0.2	0.63	1	0.8	1.45	3	0.7	1.27	3
Teratocephalus	13.4	4.70	3	12.0	6.56	9	8.2	4.01	9	6.3	4.96	9
Metateratocephalus	5.3	3.73	3	* 1.4	1.82	6	1.4	1.03	8	1.7	1.66	7
Eumonhystera	0.0	0.00	0	3.0	3.44	7	1.3	1.05	8	3.5	3.55	8
Plectus	19.8	8.41	3	21.2	3.84	9	15.6	5.32	9	12.7	4.90	9
Wilsonema	14.1	0.98	3	*** 3.6	1.51	9	12.6	6.88	9	* 5.7	4.48	9
Prismatolaimus	0.0	0.00	0	0.1	0.32	1	0.4	0.79	2	0.5	0.63	4
Eudorylaimus	5.3	2.09	3	3.7	1.48	9	2.2	2.28	7	2.6	2.47	8
Tylolaimophorus	1.5	2.67	1	0.5	0.73	4	0.9	0.91	5	1.9	1.25	8

[1)] number of sampling dates on which genus was found in each treatment x replicates; maxima are 3, 9, 9, 9 respectively; *=0.05>p≥0.01; **=0.01>p≥0.001; ***=p<0.001.

Table 3.4). The opposite was true for *Ditylenchus*, *Eumonhystera*, *Acrobeloides*, *Wilsonema* and *Aphelenchoides*.

After three years, community analyses of the nematode fauna in the mineral layer did not show effects of liming (Fig. 3.2), although *Acrobeloides* and *Drilocephalobus* had significant higher, and *Plectus* significant lower abundances in the limed plots (Table 3.4). The changes in the composition of the nematode fauna of the organic layer were significantly correlated with pH. However, up to four years after liming no change in pH was found in the 0-10 cm mineral soil (Table 3.1). Since the lime was applied on top of the organic layer, an increase of pH of the mineral soil is likely to occur first in the upper part of mineral soil. Therefore, the nematode fauna of the first 3 cm mineral soil was

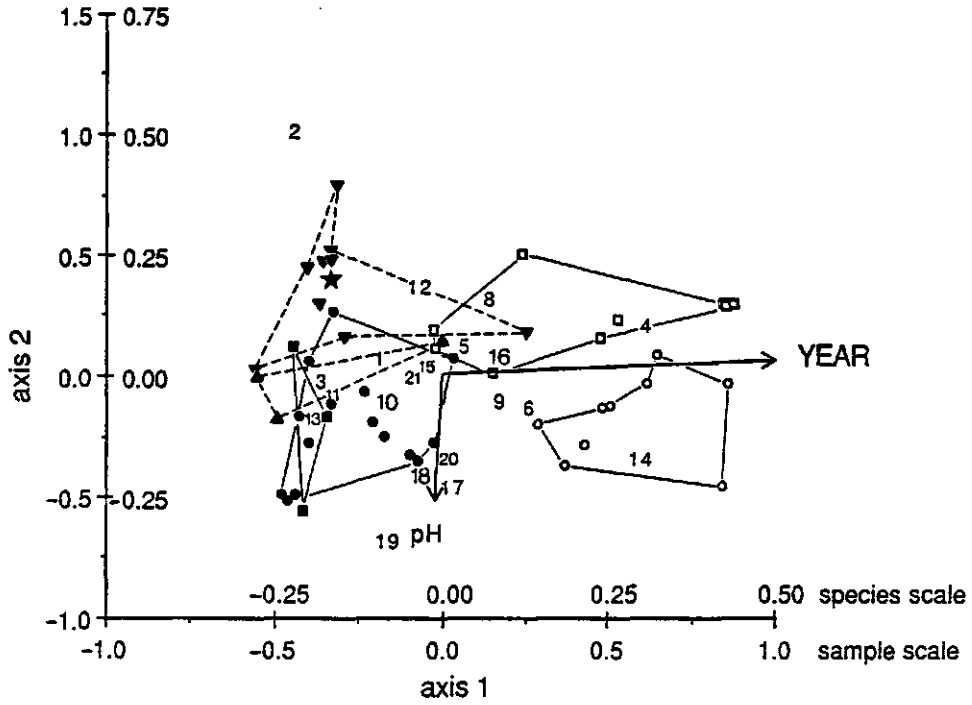

Figure 3.2. Ordination (CCA) diagram for axes 1 and 2 based on the nematode fauna of the **mineral** soil. Taxa numbers correspond to table 3.4. See fig 3.1 for explanation of symbols.

sampled in the fourth year. Table 3.1 shows that pH increased 0.7 units in the plots which received $CaCO_3$. These changes in pH were correlated with changes in the composition of the nematode fauna (Fig. 3.2). The genera *Acrobeloides* and *Eumonhystera* had significantly higher relative abundances in the limed plots, whereas *Wilsonema*, *Teratocephalus*, *Aphelenchoides* and *Steinernema* had significantly lower ones (Table 3.4).

In the plots fertilized with duck manure the only significant effect found was an increase of *Cephalenchus* (Table 3.3).

Trophic structure: Bacterial feeding nematodes were dominant both in the organic and mineral layer (Table 3.5). Predatory nematode taxa were not found. The only significant difference in trophic structure between the plots due to liming, was an increased number of omnivores and bacterial feeding nematodes in the mineral layer after four years. The same trend, although not significant, was already apparent in the previous year. In the organic layer as well as in the mineral soil hyphal feeding nematodes made up a larger part of the nematode fauna in 1989 compared with 1988, this at the expense of the root-

Table 3.3. Effect of duck-manure on the relative numbers (mean %) and frequency (f[1)]) of the dominant nematode taxa in the organic layer and mineral soil, three years after first fertilization.

GENUS	ORGANIC LAYER						MINERAL SOIL					
	CONTROL			MANURE			CONTROL			MANURE		
	mean	s.d.	f	mean	s.d.	f	mean	s.d.	f	mean	s.d.	f
Filenchus	8.1	2.78	3	12.5	2.70	3	17.2	5.87	3	17.9	5.66	3
Cephalenchus	0.0	0.00	0	0.0	0.00	0	1.3	1.52	2	* 4.9	1.60	3
Tylenchorhynchus	0.0	0.00	0	0.0	0.00	0	0.0	0.00	0	0.7	0.48	3
Ditylenchus	0.6	0.53	2	0.9	0.93	2	0.6	1.11	1	1.0	0.68	3
Aphelenchoides	20.1	15.29	3	19.1	3.17	3	14.5	8.46	3	18.4	3.10	3
Protorhabditis	2.5	1.92	3	1.9	0.04	3	1.0	1.67	1	1.1	0.96	2
Bunonema	1.6	2.72	1	1.9	0.94	3	0.0	0.00	0	0.0	0.00	0
Heterocephalobus	3.8	5.00	2	2.6	4.44	1	0.3	0.56	1	0.7	0.18	3
Acrobeloides	3.4	1.45	3	4.1	1.53	3	9.4	3.87	3	11.2	3.57	3
Cervidellus	2.8	2.48	2	3.1	2.15	3	24.5	9.56	3	14.5	6.62	3
Drilocephalobus	0.0	0.00	0	0.6	1.07	1	5.6	6.61	2	6.0	1.38	3
Teratocephalus	6.2	4.61	3	11.7	5.32	3	5.5	3.88	3	7.7	3.03	3
Metateratocephalus	4.7	0.98	3	3.4	3.53	3	0.6	1.11	1	0.1	0.17	1
Eumonhystera	0.3	0.53	1	0.6	1.11	1	0.0	0.00	0	0.3	0.36	2
Plectus	20.2	2.16	3	24.1	3.83	3	3.5	2.76	3	4.2	1.45	3
Wilsonema	9.3	4.78	3	4.1	3.24	3	2.9	0.05	3	2.6	0.37	3
Prismatolaimus	0.0	0.00	0	0.0	0.00	0	0.0	0.00	0	0.3	0.31	2
Eudorylaimus	4.0	1.39	3	4.4	3.48	3	1.9	2.54	2	0.4	0.17	3
Tylolaimophorus	3.7	2.52	3	1.6	0.52	3	5.5	3.50	3	2.3	0.70	3
Steinernema	0.0	0.00	0	0.0	0.00	0	1.6	1.51	2	1.3	0.61	3

[1)] number of sampling dates on which genus was found in each treatment x replicates; maxima are 3, 3, 3, 3 respectively; *=$p<0.05$.

feeding nematodes. This was mainly due to shifts in relative abundance of *Filenchus* and *Ditylenchus*.

Although significant effects of liming on bacterial feeding taxa were found, no differences were found when the bacterial feeding nematodes were taken as a group.

No significant changes in trophic structure could be detected in response to the application of duck manure.

Table 3.4. Effect of lime on the relative numbers (mean %) and frequency (f[1)]) of the dominant nematode taxa in the mineral soil[2)], three and four years after first applications of treatments.

GENUS		3 YEARS						4 YEARS					
	C T	- LIME			+ LIME			- LIME			+ LIME		
		mean	s.d.	f	mean	s.d.	f	mean	s.d.	f	mean	s.d.	f
1 *Filenchus*	- P	22.2	4.39	3	16.8	7.24	9	12.8	9.32	9	6.5	5.09	9
2 *Cephalenchus*	- P	0.0	0.00	0	0.1	0.20	1	0.0	0.00	0	0.1	0.20	1
3 *Tylenchorhynchus*	- P	2.1	1.93	2	0.4	0.58	4	0.1	0.28	1	0.0	0.00	0
4 *Ditylenchus*	2 H	0.9	0.93	2	0.5	1.04	2	12.5	11.33	8	8.8	4.51	9
5 *Aphelenchoides*	2 H	15.8	8.55	3	18.4	8.98	9	24.6	4.32	9	* 18.5	5.21	9
6 *Protorhabditis*	1 B	0.9	0.90	1	0.9	0.73	4	1.5	1.86	7	3.8	1.82	9
7 *Bunonema*	1 B	0.0	0.00	0	0.0	0.00	0	0.0	0.00	0	0.1	0.27	2
8 *Heterocephalobus*	2 B	0.6	1.08	1	0.1	0.32	1	0.3	0.71	2	0.9	1.33	5
9 *Acrobeloides*	2 B	2.8	2.49	3	* 12.4	5.21	9	11.6	6.62	9	*** 24.5	5.67	9
10 *Cervidellus*	2 B	23.8	6.06	3	18.0	4.29	9	9.9	5.39	9	9.9	2.90	9
11 *Drilocephalobus*	2 B	3.1	1.44	3	* 8.5	4.08	9	0.9	1.11	5	1.1	1.05	6
12 *Teratocephalus*	3 B	2.1	1.05	3	3.5	2.53	7	6.6	5.03	9	** 1.9	1.73	8
13 *Metateratocephalus*	3 B	1.5	1.42	2	0.5	0.66	5	0.0	0.00	0	0.1	0.43	1
14 *Eumonhystera*	1 B	0.0	0.00	0	0.3	0.62	3	0.7	0.63	6	* 2.8	2.61	9
15 *Plectus*	2 B	5.5	3.16	3	* 2.7	1.34	9	3.2	2.15	9	3.0	1.82	9
16 *Wilsonema*	2 B	2.8	0.94	3	3.3	1.29	9	7.4	2.48	9	* 4.7	2.63	9
17 *Prismatolaimus*	3 B	0.6	0.53	2	2.7	2.41	8	0.5	0.99	3	1.9	1.88	8
18 *Eudorylaimus*	4 O	1.2	1.37	2	1.4	1.13	6	0.5	0.74	4	1.2	0.65	8
19 *Tylencholaimus*	4 H	0.9	0.93	2	0.4	0.68	3	0.0	0.00	0	0.3	0.56	3
20 *Tylolaimophorus*	3 H	11.2	14.54	3	6.2	4.03	8	4.4	2.48	8	7.5	4.71	9
21 *Steinernema*	- I	0.9	1.62	1	1.7	1.48	8	1.7	1.28	7	* 0.5	0.59	5

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Table 3.5. Effect of lime on trophic group distribution, %Secernentia, %Rhabditida, Maturity Index (MI) and c-p value group distribution in the organic layer and mineral soil, three and four years after the first applications of treatments.

	ORGANIC LAYER				MINERAL SOIL			
	1988		1989		1988		1989	
	-lime n=3	+lime n=9	-lime n=9	+lime n=9	-lime n=3	+lime n=9	-lime n=9	+lime n=9
plant feeding	15.3	10.4	9.3	7.2	24.6	18.0	12.9	6.9
hyphal feeding	19.6	21.6	32.2	30.1	28.8	25.5	41.4	35.2
bacterial feeding	58.2	61.5	55.7	58.8	44.4	53.3	43.0 **	55.2
omnivores	5.3	3.7	2.2	2.6	1.2	1.5	0.5 *	1.2
insect parasites	0.0	0.0	0.2	0.0	0.9	1.7	1.7	0.8
% Secernentia	58.8	66.6	66.4 **	72.2	75.9	80.9	80.9	76.6
% Rhabditida	24.7 *	34.1	25.9 **	36.3	34.9	44.0	30.9 *	42.5
MI	2.36 ***	2.12	2.06	2.01	2.26	2.20	2.13	2.09
c-p 1	0.7 ***	13.0	12.1 *	16.5	1.2	1.6	2.6 ***	7.4
c-p 2	68.5	66.2	73.1	68.7	74.3	79.5	83.0	77.9
c-p 3	24.4	16.5	12.0	11.8	21.6	16.4	13.5	12.8
c-p 4	6.4	4.4	2.8	3.0	2.8	2.4	0.9 *	1.7
c-p 5	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1

*=0.05>p≥0.01; **=0.01>p≥0.001; ***=p<0.001.

◂*Legend* Table 3.4. [1] number of sampling dates on which genus was found in each treatment x replicates; maxima are 3, 9, 9, 9 respectively; [2] samples taken from 0-10 and 0-3 cm for respectively 1988 and 1989; C: c-p score of genus used to calculate the MI, plant feeding and insect parasitic genera were excluded from the calculation of the MI; T: trophic group, P plant feeding, H hyphal feeding, B bacterial feeding, O omnivores, I insect parasites; significant differences between relative abundances of +lime and -lime plots are shown for each year separately: *=0.05>p≥0.01, **=0.01>p≥0.001, ***=p<0.001.

Community indices: The percentage Secernentia (=Tylenchida + Rhabditida) in the organic layer increased from 59 in 1988 to 66 in 1989, in the untreated control plots (Table 3.5). Liming resulted after four years in a further and significant increase up to 72%. The percentage Secernentia in the mineral layer was higher than in the organic layer, but was not significantly affected by liming. In both years a significant higher percentage Rhabditida was found in the organic layer of the limed plots. In the mineral soil a significant increase was found only after four years.

In the organic layer of the limed plots a significant reduction of the MI was observed after three years, as compared to the plots without lime (Table 3.5). The MI in the plots without lime decreased from 2.36 in 1988 to 2.06 in the next year, and the differences between plots with and without lime had then disappeared. The changes in MI were due to an increase of nematode taxa with a c-p score 1 (Table 3.5). In the mineral layer no differences in MI between the plots with and without lime were found, although after four years the nematode taxa with c-p score 1 increased significantly in the limed plots. However, at the same time, c-p group 4 increased in the limed plots as well, resulting in similar MI values. The results obtained from the MI and the percentage Rhabditida largely parallel, which can be explained by the fact that all families with c-p score 1 (except the Monhysteridae) belong to the Rhabditida.

The only effect, although small, of application of duck manure was a significant decrease of the MI of the mineral layer, from 2.22 to 2.14.

C-P triangles

Harderwijk Forest Fertilization Project: In Fig. 3.3 the c-p triangles for the Harderwijk forest are given. In the graphs the proportion of c-p group 1, c-p group 2, and the sum of the c-p groups 3, 4 and 5 are given on a side of the triangle. In both years, in the organic layer liming resulted in increased numbers of c-p group 1, whereas the relative abundance of c-p group 3-5 was not altered (Figs 3.3.a and 3.3.b). After three years, c-p group 1 composed only a relative insignificant part of the nematode fauna of the mineral layer (Fig. 3.3.c). One year later the proportion of c-p group 1 increased in the $CaCO_3$ treatments, but remained low in the control (Fig. 3.3.d).

Other forest fertilization projects: Fig. 3.4 shows c-p triangles of nematode assemblages in a number of forest fertilization, liming and acidification experiments in Scandinavia and Central Europe. The selection of experiments is confined to field studies of coniferous forests, in which full taxa lists are given. The fertilization and liming experiments can be divided into applications of urea, ash and mineral calcium compounds ($CaCO_3$, CaO and $Ca(OH)_2$), all three resulting in an increased soil pH. In the

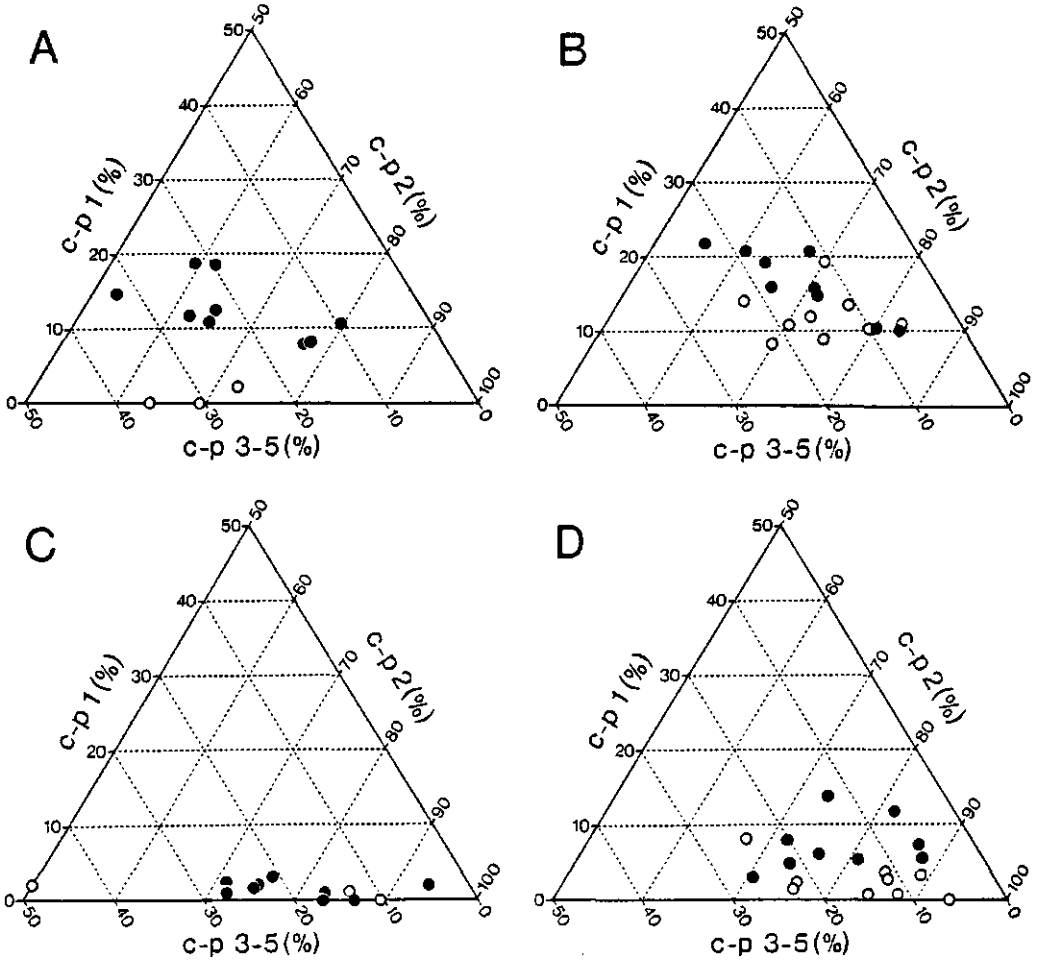
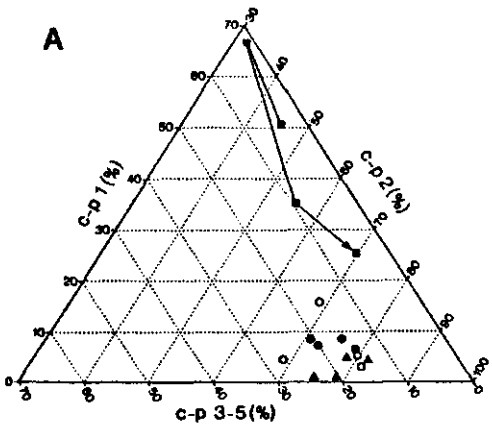
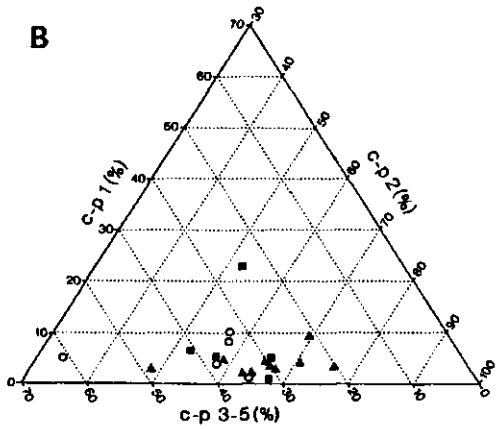
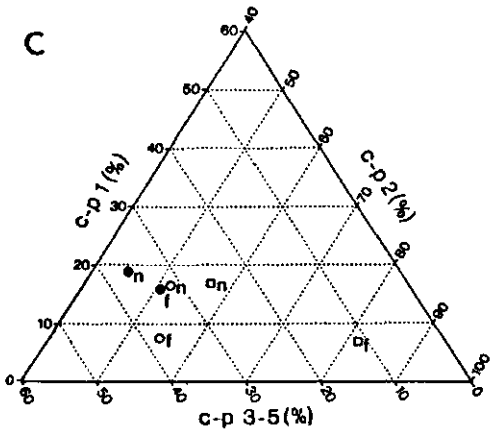
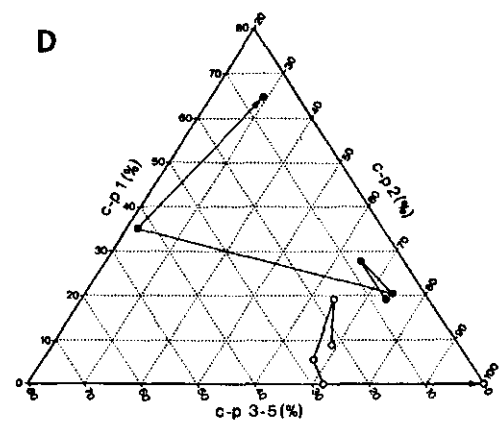


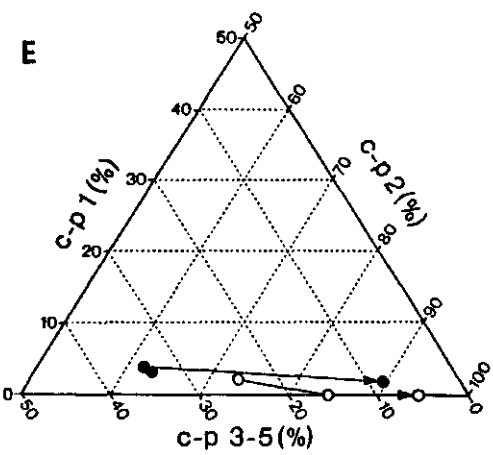
Figure 3.3. C-P triangles for the organic layer 1988 (A) and 1989 (B) and for the mineral soil 1988 (C) and 1989 (D) of Harderwijk forest (-lime: ○, +lime: ●). Note that only a section of the total triangle is shown.

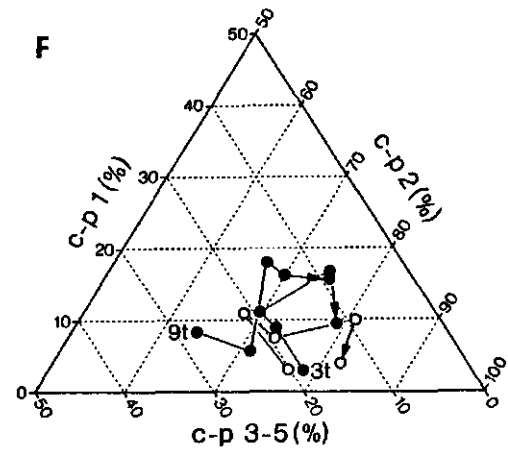

A
c-p 1(%)
c-p 2(%)
c-p 3-5(%)


B
c-p 1(%)
c-p 2(%)
c-p 3-5(%)


C
c-p 1(%)
c-p 2(%)
c-p 3-5(%)


D
c-p 1(%)
c-p 2(%)
c-p 3-5(%)


E
c-p 1(%)
c-p 2(%)
c-p 3-5(%)


F
c-p 1(%)
c-p 2(%)
c-p 3-5(%)
9t
3t

acidification experiments at Fexboda and Norrliden, H_2SO_4 was used to lower soil pH.

In samples taken within the first three months after liming with calcium compounds no shifts in c-p value group distribution occurred (Figs 3.4.a and 3.4.f). However, four, seven and twelve months after liming the proportion of c-p group 1 was higher (Fig. 3.4.f). A comparable increase was also found in other experiments two and six years after liming (Figs 3.4.c respectively 3.4.d).

In Tiefensee (Fig. 3.4.e) and Norrliden (Fig. 3.4.c), respectively one and twelve years after liming, the proportion of c-p group 3-5 in the limed plots was higher than in the corresponding controls.

Fertilization with urea resulted in a clear short-term shift in c-p value group distribution (Figs 3.4.a and 3.4.b). One month after the start of the urea fertilization the proportion of c-p group 1 increased to 50% and the proportion of c-p group 3-5 dropped below 10% (Fig. 3.4.a). After a further increase to 67% in the next month, c-p group 1 subsequently decreased to a level of 25% four months after the start of the experiment. No recovery of c-p group 3-5 could be observed. During this four months period the MI of the urea treated plots was respectively 1.55, 1.36, 1.79 and 1.84, which is less than in the control (average MI is 2.20). The second year after application of urea, another experiment showed no differences in c-p value group distribution (Fig. 3.4.b).

The first year after application of ashes no differences in c-p value group distribution were found (Fig. 3.4.a), whereas after one year a decrease of c-p group 3-5 possibly occurs (Fig. 3.4.b).

Artificial acidification also resulted in a relative decrease of c-p group 3-5 (Fig. 3.4.c) which was most obvious in Fexboda where the final application of H_2SO_4 was 6 months

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◂*Legend* Figure 3.4. C-P triangles for forest fertilization experiments A: Scots pine forest at Ruotsinkylä (Hyvönen & Huhta 1989; sampling at monthly intervals, 1-4 months after first fertilization, B: Scots pine forest at Tammela (Hyvönen & Huhta 1989; sampling at monthly intervals, 12-16 months after first fertilization), C: Norway spruce forest at Fexboda (sub-script "f") and Scots pine forest at Norrliden (sub-script "n") (Hyvönen & Persson 1990; sampling at respectively two and twelve years after first applications), D: spruce forest at Hermsdorf (Bassus 1960; sampling during the 6th and 7th year after liming), E: spruce forest at Tiefensee (Bassus 1960; sampling at III, V and X, one year after liming), F: Scots pine forest at De Peel (Schouten unpubl.; sampling at one week before and six weeks respectively 4, 7 and 12 months after liming).

Applications are indicated as ■: urea compounds, ●: Ca compounds, ▲: ashes, □: H_2SO_4, ○: controles. In some triangles, time-series are connected with solid lines, in which the arrow indicates the last sampling occasion. In figure F the applied dose of lime (3 or 9 ton ha^{-1}) is indicated at the beginning of each time-series of samples, and figures shown are means of two replicate fields.

before sampling. Seven years after final artificial acidification only a relatively small decrease of c-p group 3-5 was visible (Norrliden), possibly indicating some recovery.

From an analyses of literature data and our own data, it can be concluded that the c-p value group distribution can be helpful in analyzing effects of disturbances like forest fertilization. Fertilization with urea leads to dramatic short term (weeks) shifts in the composition of the nematode fauna (increase of c-p group 1 and decrease of c-p group 3-5), which seem to last only for several months.
Shifts in the composition of the nematode fauna due to liming with calcium compounds (increase of c-p group 1) appear after months, and can be still demonstrated after several years. Artificial acidification is found to lead to a relative decrease of c-p group 3-5.

DISCUSSION

Effects of lime on nematode fauna

Forest liming is thought to result into increased activity of bacterial populations (reviewed by Persson 1988) and therefore changes within bacterial feeding nematode populations can be expected. Three and four years after the first application of lime at Harderwijk, no increase of the bacterial feeding nematode population as a whole was found in the organic layer. However, the proportions of the bacterial feeders *Acrobeloides*, *Protorhabditis* and *Eumonhystera* were increased, and their absolute numbers were 2.3 to 5.5 times higher than in the plots which did not receive lime. An increase in density of *Acrobeloides*, species of the Rhabditidae and, if present *Eumonhystera*, is found in most forest fertilization experiments where fertilizers caused decreasing acidity (Hyvönen & Huhta 1989, Bassus 1960, 1967, Ratajczak *et al.* 1989). The Rhabditidae and *Eumonhystera* are considered to be r-strategists (*sensu lato*; c-p value 1) characterized *inter alia* by short generation times and numerous progeny (Bongers 1990b). Their occurence, as well as that of *Acrobeloides*, is found to be positively correlated with the productivity of bacteria populations (Schiemer 1983, Sohlenius 1973).

In the same experiment at Harderwijk Kuyper & De Vries (1990) found a decreased diversity and abundance of litter decomposing fungi in the limed plots. The total number of hyphal feeding nematodes was not significantly influenced by liming. However, the relative abundance of the hyphal feeding genus *Ditylenchus* was lower in the limed plots. It should be noticed that in some cases it appeared to be difficult to distinguish juvenile plant-parasitic *Filenchus* species (*Filenchus* cf *ditissimus*; trophic group classification according to Yeates *et al.* (1993a)) from juveniles of morphologically very similar

Ditylenchus species. In addition, four years after liming (i.e. the first year that significant differences in the composition of the nematode fauna were observed in the mineral soil due to liming) the relative abundance of the dominant hyphal feeder *Aphelenchoides* was significantly reduced in the mineral soil of the limed plots.

These findings support the general observation that a decreased acidity results in conditions favouring bacteria relative to fungi (Alexander 1977, Swift *et al.* 1979, Persson 1988). Huhta *et al.* (1986) concluded that it seems to be plausible that the decrease of acidity alone is enough to explain the changes in soil fauna composition. Our results do not refute this idea: changes in the nematode taxa given above are found after liming only, and only when bulk soil pH has been increased.

Although no species were found to become extinct after bulk soil pH increased by two or more units, the diversity found here might not be the historical potential richness of the nematode fauna. Since no pre-pollution surveys on Dutch nematode communities exist, the potential richness of the nematode fauna in these environments is unknown.

Maturity Index and c-p triangle

Interpretation of differences in relative distribution of the c-p groups for the above mentioned experiments, revealed changes and trends in situations where no change in MI could be found. Most of the MIs of the coniferous forests in which fertilization experiments were carried out, lay between 1.9 and 2.5. Within this range of maturity indices an arbitrary MI can come about by a wide range of different c-p value group distributions (Chapter 2). For instance, two samples with comparable MI may differ significantly in relative distribution of r- and K-strategists (*sensu lato*). Reduction of a nematode taxa list into a c-p value group distribution based on autecological characteristics of the nematode species, still reveals effects of environmental disturbances on the nematode fauna. But a further reduction of this information into a MI can thus obscure more or less gradual effects on the nematode communities. Acute effects like those caused by application of urea, which result in a nematode community with c-p value group 1 and c-p group 3-5 of respectively >20 and <10 %, will lead to maturity indices ≤ 1.8. In general, such low maturity indices indicate environmental disturbances.

Response time of the nematode fauna

Although both fertilization with urea or lime seem to result in an increased proportion of nematode taxa with low c-p scores, the times of first response, intensity, and duration of response, appear to differ significantly. Application of urea results in strong, short-term changes in the nematode community (Huhta *et al.* 1986), whereas effects of liming with calcareous compounds are observed not until several months, but they seem to last for several years. Relatively rapid shifts in pH, enrichment with nitrogen or input of dead

organic material (e.g. because of die back of mosses) can be responsible for the effects of urea (Huhta *et al.* 1986). The gradual shifts in nematode community composition after liming with calcareous compounds agree with the relatively low solubility rate of calcium carbonate. In contrast to urea the increase in soil pH lasts for decades and effects on pH in the mineral soil are, compared to the organic layer, delayed (Nihlgård *et al.* 1988). The same was found for effects of liming on the composition of the nematode fauna in our experiment and in similar experiments in Dutch Scots pine forests (Schouten *et al.*, in prep).

In our experiment, effects due to differences in sampling depth between the years cannot be excluded, but are thought to be of minor importance. First changes in nematode fauna composition are expected initially to occur in the top centimetres of the mineral soil. As nematode population density decreases with soil depth, changes in nematode fauna composition three years after liming, when 0-10 instead of 0-3 cm mineral soil was sampled, should have been noticed.

State of the nematode fauna

Comparison of the average c-p group 4-5 scores, i.e. the proportion of extreme K-strategists (*sensu lato*), of the control plots from two fertilized Scots pine forests from the Netherlands (this study, Schouten *et al.* in prep.) with five Scots pine forests from Scandinavia (Sohlenius & Wasilewska 1984, Hyvönen & Huhta 1989, Hyvönen & Persson 1990), shows lower values for the Dutch forests: 0.7-5.8% respectively 11.7-24.4%. Comparable low values (2.1-8.1%) are found also in Dutch Scots pine forests at other locations (own observations). In addition, experiments studying effects of artificial acidification showed a decrease or disappearance of especially nematode taxa with high c-p scores (Fig. 3.4.c, Ratajczak *et al.* 1989, Schouten & Van der Brugge 1989). If these taxa with high c-p scores are part of the potential fauna, the absence of these taxa possibly coincides with the decline of Dutch forests on sandy soils.

Although in Harderwijk, no new nematode taxa were noticed in the limed plots, within four years of liming. Floristic- and myco-sociological studies on the other hand did record higher plant and fungal species in the limed treatments of Harderwijk forest, which were not found in the non-limed plots (Dirkse & Van Dobben 1988, Kuyper & De Vries 1990).

CONCLUSIONS

- Three and four years after liming, the total number of nematodes tended to be reduced in the organic layer, whereas no significant effects were found in the mineral layer.
- In the organic layer, differences in the structure of the nematode fauna were demonstrated three and four years after liming. In the mineral layer such differences were found only four years after liming. These differences only occurred when bulk soil pH(KCl) had increased.
- Liming resulted at all times, irrespective of soil layer, in a relative increase of *Acrobeloides*, *Protorhabditis* and *Eumonhystera*, and a relative decrease of *Wilsonema*.
- From the community parameters tested, only percentage Rhabditida and c-p group 1 responded significantly at all these times when changes in nematode community structure due to liming were found.
- Although significant effects of liming on bacterial feeding taxa were found, no differences were found when the bacterial feeding nematodes were taken as a group.
- C-P triangles prove to be a helpful technique in studying disturbances of the nematode fauna in soil ecosystems.
- Liming and fertilization with urea result in increased proportions of nematode taxa with c-p score 1.

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NEMATODE COMMUNITY STRUCTURE IN RELATION TO SOIL AND VEGETATION CHARACTERISTICS

SUMMARY

To study the prospect of nematodes contributing to an ecological soil classification, the nematode fauna of a variety of Dutch terrestrial habitats was studied. A total of 209 samples from 44 nature reserves or slightly managed sites (n=94) differing in vegetation (forest, shrubs, heathland, grassland) and soil type (clay, loam, sand) were studied. Nematodes were extracted from bulk soil samples taken from the 0-10 cm mineral soil, and were identified to genus. Multivariate analyses techniques were used to classify the nematode samples in Sample Groups (SG). Seven SGs could be distinguished and these could also be described by soil characteristics in combination with the vegetation: SG A grasslands, dwarf-shrub vegetation and forest gaps on sandy soils; SG B grasslands and forests on clayey soils; SG C-D deciduous forests on sandy-loam soils; SG E-F deciduous forests on sandy soils; SG G coniferous forests on sandy soils. The nematode faunae of the SGs D-G proved to be very similar, and was dominated by ten taxa: Acrobeloides, Aphelenchoides, Cephalenchus, Filenchus *A*, Filenchus *B*, Plectus *A*, Prismatolaimus, Rhabditidae, Tylolaimophorus *and* Wilsonema. *Bias due to seasonal fluctuations and techniques used, appeared small compared to differences in nematode fauna structure between different sites. The actual vegetation of some sites were not in agreement with the vegetation expected on "site characteristics". Analyses of the nematode fauna supported the observed inconsistencies between actual and expected vegetation.*

INTRODUCTION

Increasing awareness of the nature and extent of soil pollution has resulted in an increasing effort in research of the consequences of soil pollution on soil organisms and their role in soil processes. This has resulted in exploring the possibilities of biological soil assessment systems to indicate the ecological condition of a soil.

Nematodes possess many qualities which makes them suitable as bioindicator organisms for soil quality (Bongers 1990b, Freckman 1988, Samoiloff 1987). At the community level changes in the composition of the nematode fauna have been found to indicate effects of pollutants (Bongers *et al.* 1991, Cantelmo & Rao 1978, Cantelmo *et al.* 1979, Sturhan 1989, Weiss & Larink 1991), liming (Hyvönen & Huhta 1989, Hyvönen & Persson 1990, Ratajczak *et al.* 1989, Chapter 3), acidification (Hyvönen & Persson 1990, Ruess & Funke 1992), agricultural practices (Freckman & Ettema 1993) and recovery

after disturbance (Yeates *et al.* 1991, Ettema & Bongers 1993). Moreover, as free-living soil nematodes are represented at most trophic levels of the food web, they are thought to be closely connected to, and reflect, fundamental ecological processes (e.g. decomposition, mineralisation, nutrient cycling) in soils. In arable soils, changes in the composition of the nematode fauna, have been found to be related to changes in activity and/or biomass of the microflora, and in microfloral mediated processes such as nutrient dynamics and nitrogen mineralisation (e.g. Brussaard *et al.* 1990, Sohlenius *et al.* 1987, Ettema & Bongers 1993).

Effects of disturbance on fundamental ecological processes in soils are expressed by functional or structural changes in the nematode community. Analysis of the composition of the nematode fauna can serve as a basis for ecological assessment of soils (Bongers *et al.* 1989). Depending on the specific goals of the study, either direct interpretation of the taxa composition or evaluation of derived indices may be used. In monitoring studies results can be compared with earlier investigations and observed changes can be interpreted. However, the assessment of the ecological condition of soils for which no previous information is available requires a reference system. As with most indices, the results of nematological indices and their interpretation, must be related to the type of habitat studied. A reference system, which describes the ecological characteristics of soils (an ecological soil classification), may serve as a basis to assess the condition of a previously unsampled soil. If the direct aim of an ecological soil classification is to provide a reference for a biological soil assessment system, then it has advantages to base both the assessment and the reference system on the same organisms. Only then will the ecological requirements of the indicator organisms harmonize with the ecological "boundaries" between the distinguished types or classes.

Although the nematode fauna of the Netherlands is well characterized taxonomically (Bongers 1988) and about one million soil samples have been analyzed for plant parasitic nematodes, the first full species lists of terrestrial habitats appeared in the last decade. These lists (Tamis 1986a, Bongers *et al.* 1989, Manger & Schouten 1989) are mainly restricted to samples from forests and nature reserves, and hitherto, comprehensive species lists of agricultural systems are almost lacking.

Within Europe studies of the complete nematode fauna of individual natural habitats have included coniferous forest (Bassus 1960, Hyvönen & Persson 1990, Magnusson 1983a, Sohlenius & Wasilewska 1984, Ratajczak *et al.* 1989), deciduous forest (Popovici 1984, Yeates 1972 & 1973) and other habitats (Coomans 1961, Gerber 1981 & 1985, Jordana *et al.* 1987, Yuen 1966, Buttner 1989, Castillo Castillo *et al.* 1985). Studies comparing the nematode faunae of a range of terrestrial natural habitats are given by

Arpin & Ponge (1986), Bassus (1962, 1967), Bongers *et al.* (1989), Nielsen (1949) and Wasilewska (1970). Some studies comparing a range of habitats have concentrated mainly on root feeding nematodes (Baujard *et al.* 1979, Scotto La Massese & Boulbria 1980). In the U.S.A. an extensive study of the nematode fauna of different forest sites was carried out by Johnson *et al.* (1972, 1973, 1974), who assessed the nematode fauna of 18 sites with differing soils and vegetation.

Since 1985 systematic inventories have been made of the nematode fauna of lightly managed soils in the Netherlands. It was shown that habitats could be classified into clusters with specific biotic and abiotic properties on the basis of the nematode fauna (Bongers *et al.* 1989). Seasonal differences in the abundance of nematodes did not influence the classification achieved, neither did the level (species or genus) of identification. Except for a cluster of forests on nutrient poor sandy soils, the clusters of sites could be related to differences in biotic (vegetation) and abiotic (soil) properties measured at each site.

The aim of the present investigation was to study relationships between the composition of the nematode fauna of forests on sandy soils and their biotic and abiotic environment, and to indicate their position within a habitat classification such as that proposed by Bongers *et al.* (1989). Moreover a study of these soils has high priority because due to their poorly buffered nature they are among the soils most sensitive to environmental pollution and eutrophication (Gleichman-Verheijen *et al.* 1991). In addition to the 46 sites of Bongers *et al.* (1989) we use 120 samples representing 14 "native" forest types that are typical of Dutch sandy soils (Van der Werf 1991). Multivariate analyses (cluster and ordination analysis) are used to group samples with related nematode faunae. Vegetation and physical and chemical soil properties of these sample groups are described.

MATERIALS AND METHODS

Sites, sampling and isolation

In September 1985 46 sites, representing a variety of vegetation types (grassland, heather, shrubs, pine and deciduous forest and outer marshes) and soil types (clay, loam and sand), were sampled. Eight of these sites were also sampled at three-monthly intervals and one of those was sampled tenfold in May 1987. From April to May 1988 another 121 samples were collected at 48 forest sites. These were selected on the basis of the potential distribution of the forest types within the Netherlands, to represent examples of "native" forest types on sandy and sandy-loam soils (Van der Werf 1991).

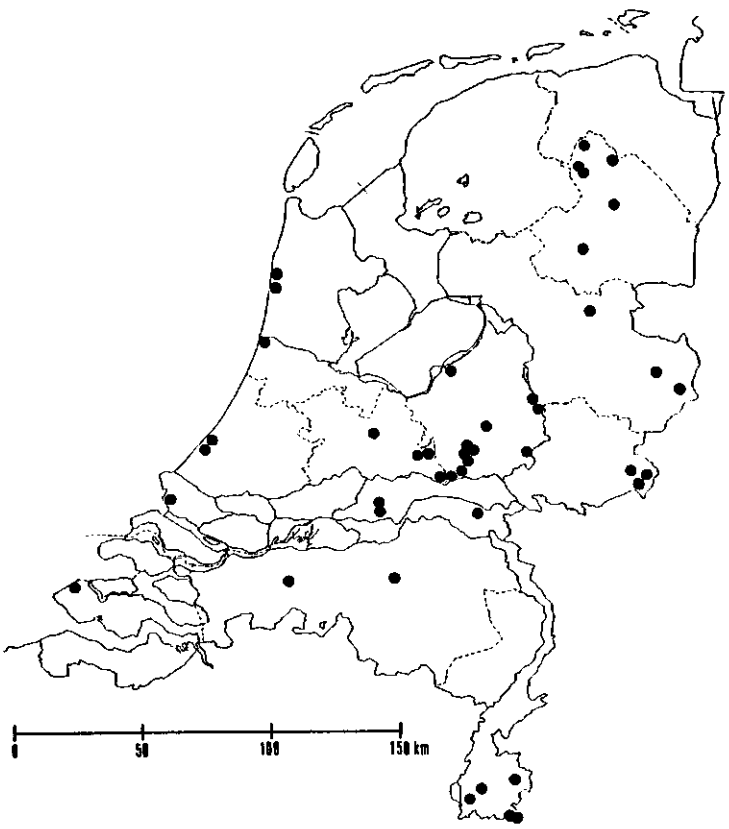

Figure 4.1. Geographical distribution of the 44 nature reserves and rural estates (●) that were studied.

In total 209 samples from 94 different sites located at 44 nature reserves and rural estates were studied (Fig. 4.1). At each site a representative area of 100 m² was selected, and 50 cores (17 mm diameter) taken from the 0-10 cm mineral soil. The cores were bulked and sub-samples were taken for nematode extraction and chemical and physical analysis. At each site sampled in 1988, 2-3 such plots were sampled. Detailed descriptions of the sites, sampling procedure and physical chemical analyses are given in Bongers *et al.* (1989) and Van der Berg *et al.* (1990). Vegetation relevés (Braun-Blanquet method) were made only of the sites sampled in 1988; the nomenclature is according to Van der Meijden *et al.* (1990). Nematode taxa lists for samples collected before 1988 are presented in Bongers *et al.* (1989), and those from 1988 are given in Appendix 1.

Nematodes were isolated using an Oostenbrink elutriator (Oostenbrink 1960), fixed, transferred to glycerin and analyzed in mass slides as described in Bongers *et al.* (1989). They were identified according to Bongers (1988). Subsequent multivariate analyses were

based on families (Rhabditidae, Qudsianematidae), genera and on the following lower taxa:

Filenchus A: small with filiform tail cf. *F. helenae*; *Filenchus* B: small with short rounded tail cf. *F. ditissimus*; *Filenchus* C: large with filiform tail cf. *F. vulgaris*; *Hemicycliophora* A: stylet <110 μm; *Hemicycliophora* B: stylet >110 μm; *Paratylenchus* A: stylet <17 μm; *Paratylenchus* B: stylet 22-33 μm; *Paratylenchus* C: stylet 44-66 μm; *Paratylenchus* D: stylet >70 μm;

Plectus A: c'>8; *Plectus* B: c'<8; *Plectus* C: cf. *P. pusillus* (long stoma);

Tripyla A: cephalic setae >0.25 cephalic diameter; *Tripyla* B: cephalic setae <0.25 cephalic diameter;

Paramphidelus A: c=3-4; *Paramphidelus* B: c=22-27;

Criconematidae (excluding *Hemicriconemoides*) species: Criconematidae A: stylet <50 μm; Criconematidae B: stylet 50-84 μm; Criconematidae C: stylet >84 μm.

We ended up with a data set giving the relative abundance of 130 nematode taxa in 209 samples.

Numerical analysis

The data set was analyzed using the clustering programme FLEXCLUS (Van Tongeren 1986) followed by detrended correspondence analysis (DCA) (Ter Braak 1987). In the analyses relative abundance was transformed as follows: 2^n% becomes (n+1)%, with n=0-7. In both programmes the option 'downweighting of rare species' was used. These procedures served to normalize the distribution of relative abundance and eliminated effects of chance occurrence of rare species. In DCA, detrending by second-order polynomials was used.

Successive detrended correspondence analyses were necessary to interpret the structure of the data set. Each sequential analysis was primarily based on the same data set, but from which distinctive groups of samples (Sample Groups) detected in the graph of the first and second axes of earlier analyses were excluded (Peet 1980, Verdonschot 1990). Sample groups (SG) are composed of one or more FLEXCLUS clusters from which the site scores for the DCA axes 1 and 2 do not differ significantly; such SGs are based on the quantitative composition of the nematode fauna in each sample. Analysis of variance was used to test for significant differences.

To characterize the nematode fauna of the SGs, the typifying weight (Verdonschot 1984, 1990) of the taxa was calculated for each SG. This typifying weight is derived from constancy, fidelity and concentration of abundance estimates (see Table 3.4 page 65 in Verdonschot (1990)). Constancy is the number of occurrences of a taxon in a SG divided by the number of sites of that SG; fidelity is the ratio of constancy of a taxon and its

overall relative frequency; concentration of abundance is the average abundance of a taxon in a SG divided by its average overall abundance.

Differences in chemical and physical characteristics of soils representing the SGs (based on nematode faunae) were tested using the Mann-Whitney U-test with $p < 0.05$.

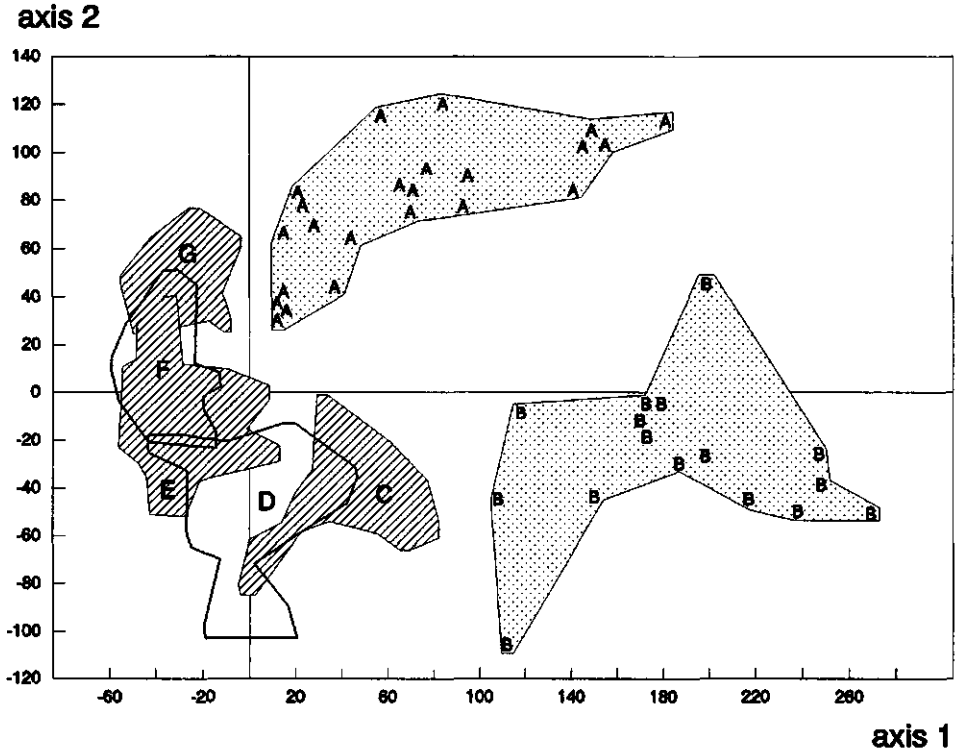

Figure 4.2. Detrended correspondence analysis diagram for the 209 nematode samples. Samples are represented by letters corresponding to the Sample Group (SG) to which they belong. The contour lines describe the total variation of each SG. For clarity the SGs C-G are represented only by their contour lines. See text for characteristics of the SGs.

RESULTS

Multivariate analyses

Using FLEXCLUS, 22 different clusters could be distinguished. By two subsequent DCA, these 22 clusters were combined into seven Sample Groups (Figs. 4.2 and 4.3). The SGs A (containing 23 samples) and B ($n=16$) split off on axis 1 and 2 of the first DCA (Fig. 4.2). The eigenvalues of these axes were 0.303 and 0.157 respectively. The third axis had an eigenvalue of 0.089. Based on the first two axis of the second DCA, the

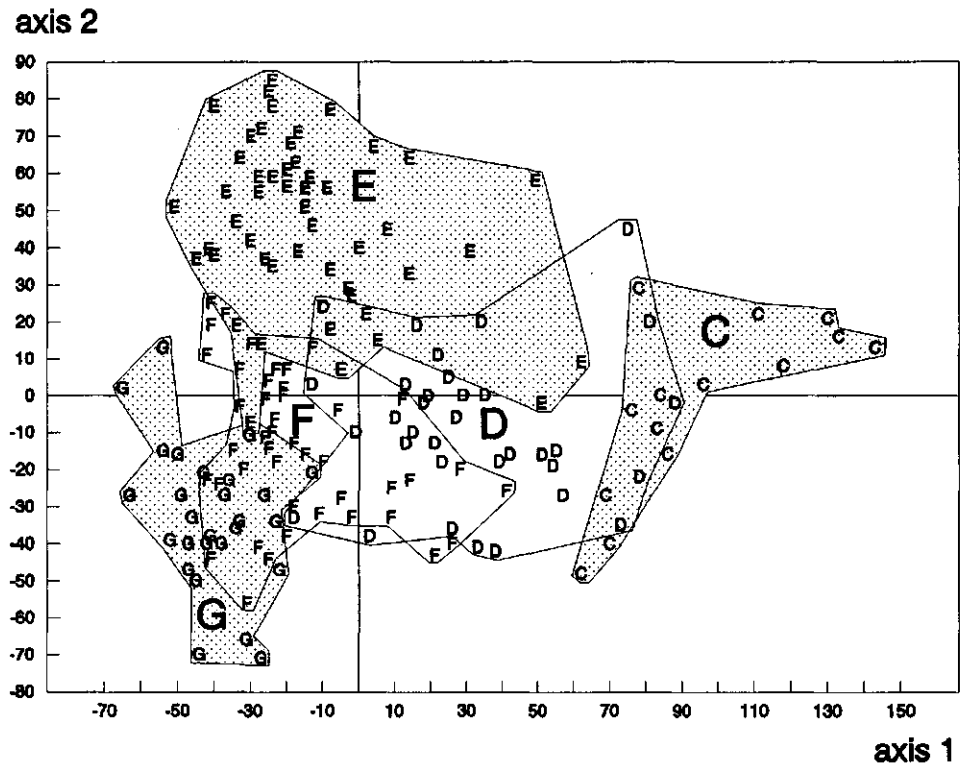

Figure 4.3. Detrended correspondence analysis diagram for the 170 samples of the Sample Groups C-G. The samples of the SGs A and B were not included in the analysis. See Fig. 4.2 for details. Note that scales differ from those in Fig. 4.2.

remaining samples (n=170) were sub-divided into another five SGs (Fig. 4.3). The eigenvalue of the first three axes of the second DCA was 0.159, 0.106 and 0.080 respectively.

Sample Group A can be characterized as grass and dwarf-shrub vegetation and forest gaps on sandy soils. SG B comprises of grasslands and forests on mainly clayey soils. The SGs C-D and E-G are made up of forests on sandy-loam and sandy soils respectively, and silt fraction decreases in the sequence C-G. SGs C-F mainly comprises of deciduous forests, whereas most of the sites of SG G are coniferous forest.

Table 4.1 gives typifying weights and relative abundances of the common taxa for each SG. Detailed soil chemical and physical characteristics of the seven SGs and their floristic descriptions, are given in Tables 4.2-4.4.

Nematode taxa

In Table 4.1 the occurrence of 43 nematode taxa with a constancy ≥0.50 in at least one SG is given. Highly typical taxa (i.e. typifying weight >9) occur only in SGs A, B and C. These SGs differ greatly from other SGs as could be seen in the DCAs, where they occupied the most extreme positions (Figs. 4.2 and 4.3). The nematode faunae of the SGs D-G, however, are similar. Ten taxa (*Acrobeloides*, *Aphelenchoides*, *Filenchus* A, *Filenchus* B, *Tylolaimophorus*, *Cephalenchus*, *Wilsonema*, *Prismatolaimus*, *Rhabditidae* and *Plectus* A) are present in at least 70% of the samples of the SGs D-G. Moreover, 79, 98, 95 and 96% of the samples of respectively SG D, E, F and G have nematode faunae consisting of more than 50% of these ten taxa.

Typical taxa for SG B are *Neopsilenchus*, *Boleodorus*, *Paratylenchus* B, *Pro/Mesodorylaimus*, *Anatonchus*, *Pungentus*, *Mylonchulus*, *Tripyla* A, *Anaplectus*, *Aphelenchus* and *Coslenchus*. Their occurrence in the other SGs is always at constancy <0.30 and mainly restricted to SGs A and C. *Basiria* and *Cephalobus* are also highly typical for SG B, but they occur in other SGs with constancies >0.30. The nematode faunae of SG B (grasslands and forests on clayey soils) were most similar to SGs A and C (Fig. 4.2). Genera with maximum occurrence in SGs B and C are *Cephalobus*, *Heterocephalobus* and *Rotylenchus* (Table 4.1) and they were present in forests as well as in grasslands. On the other hand, Qudsianematidae, *Wilsonema*, *Steinernema* and *Cervidellus*, which were common in all other SGs, were almost lacking in SG's B and C. *Pratylenchus* and *Panagrolaimus* have their highest constancy in the SGs A and B (Table 4.1). *Pratylenchus* was present in both forests and grasslands, whereas *Panagrolaimus* occurred only in grasslands and one Scots Pine forest (with an unbroken herb layer of *D. flexuosa*). *Plectus* B is the only taxon typical for SG A, B and C together.

The nematode fauna of the forested sites reflects gradual changes going from SG C via D to SG E or to the SGs F and G (Fig. 4.3). *Rotylenchus*, *Alaimus*, *Diphtherophora*, *Plectus* C and Trichodoridae are highly typical taxa of SG C (Table 4.1). The characteristic taxa of the SGs B and C are absent or show a decreasing constancy in the SGs D-G, whereas other taxa (e.g. *Cephalenchus*, *Tylolaimophorus*, *Acrobeloides*, *Wilsonema*, *Steinernema* and *Cervidellus*) show an opposite trend.

SG E is an exception to the trend shown in Fig. 4.3. Some taxa (*Drilocephalobus*, *Monhystera*, *Prismatolaimus*, Qudsianematidae and *Plectus* A) which are important in the

Legend Table 4.1. [1)] taxa with constancy ≥50%; the total number of sites are given between brackets.

Table 4.1 Typifying weight and frequency of occurrence (Constancy; %) of the common[1)] nematode taxa for the seven Sample Groups.

TAXON	TYPIFYING WEIGHT							CONSTANCY						
	A	B	C	D	E	F	G	A (23)	B (16)	C (14)	D (34)	E (50)	F (44)	G (28)
Paratylenchus A	12	1	0	1	1	1	0	70	13	0	3	2	4	0
Panagrolaimus	11	2	1	1	1	0	1	57	38	7	3	8	0	4
Aglenchus	12	1	1	1	1	1	1	52	13	29	3	4	2	4
Pratylenchus	9	9	1	1	1	1	1	57	63	14	9	4	11	11
Plectus B	9	3	3	1	1	1	1	96	63	71	24	12	11	25
Paratylenchus B	1	12	0	1	1	0	0	22	75	0	6	6	0	0
Boleodorus	1	12	0	0	1	0	0	22	69	0	0	2	0	0
Pungentus	2	11	0	0	0	0	0	26	50	0	0	0	0	0
Neopsilenchus	0	12	1	0	1	0	1	0	69	7	0	2	0	4
Coslenchus	1	11	1	0	0	0	1	4	50	7	0	0	0	4
Basiria	1	12	2	1	1	0	0	13	81	36	3	2	0	0
Rotylenchus	0	5	12	2	1	1	0	0	63	100	38	6	22	0
Plectus C	0	0	11	1	0	1	1	0	0	50	6	0	2	11
Pro/Mesodorylaimus	1	12	1	1	1	1	0	22	94	29	3	4	2	0
Cephalobus	2	12	5	1	1	1	1	39	100	64	18	12	2	7
Alaimus	2	1	12	1	1	1	1	39	25	57	18	10	7	4
Diphtherophora	1	1	12	1	1	0	1	22	19	57	3	6	0	4
Heterocephalobus	2	9	3	1	1	1	1	48	75	71	44	10	27	18
Rhabditidae	1	3	3	1	1	3	1	74	94	100	79	62	78	75
Malenchus	1	1	3	1	1	1	3	17	31	71	53	56	47	61
Trichodoridae	2	1	9	1	1	1	2	26	6	71	26	18	18	29
Tylencholaimus	5	1	5	1	1	1	2	48	6	57	9	10	27	32
Helicotylenchus	5	1	1	2	1	1	1	52	19	36	38	10	20	25
Filenchus C	1	5	1	6	1	1	1	4	81	64	65	18	40	14
Criconematidae C	1	1	3	3	3	3	1	9	19	79	76	58	62	7
Paratylenchus C	0	1	6	3	1	1	0	0	13	71	65	20	22	0
Cephalenchus	1	1	1	3	3	3	1	9	6	64	91	90	98	7
Drilocephalobus	0	1	1	1	1	5	1	0	6	14	41	22	51	7
Monhystera	1	0	1	1	1	5	2	4	0	21	50	14	84	43
Tylolaimophorus	1	0	1	1	3	3	3	35	0	64	68	78	93	75
Filenchus A	1	1	3	3	3	3	1	70	6	100	91	88	100	64
Filenchus B	1	1	1	3	3	1	1	43	69	79	97	96	100	86
Acrobeloides	3	1	1	1	3	1	3	100	63	50	100	100	100	100
Aphelenchoides	1	1	1	1	3	1	3	87	75	100	94	98	98	96
Ditylenchus	1	1	1	1	3	1	1	65	75	79	56	90	58	57
Teratocephalus	1	1	1	1	1	3	3	52	19	57	41	56	93	68
Prismatolaimus	1	1	3	1	1	3	3	70	50	86	85	40	93	93
Metateratocephalus	1	1	1	1	1	3	3	57	13	79	44	54	96	82
Qudsianematidae	3	1	1	1	1	3	3	65	44	29	74	54	73	79
Wilsonema	1	1	1	1	1	3	3	65	13	29	56	64	96	93
Steinernema	3	1	1	1	3	3	3	61	13	14	26	68	71	71
Cervidellus	1	0	1	1	1	3	3	57	0	21	24	52	53	75
Plectus A	1	1	1	1	1	3	6	43	6	57	74	40	91	100

D-F-G sequence, have exceptionally low constancies in SG E (Table 4.1). On the other hand *Ditylenchus* reaches maximum constancy (0.90) in SG E, whereas its constancy in SGs D, F and G is less than 0.58.

Plectus A, *Wilsonema* and *Prismatolaimus* reach maximum constancies in the forested sites on sandy soils (SGs F and G). Despite great similarity in composition of the nematode faunae of these SGs, some clear differences exist. For example *Tylolaimophorus, Teratocephalus, Metateratocephalus, Monhystera* and *Drilocephalobus* have maximum constancy in SG F. *Drilocephalobus, Criconematidae C* and *Cephalenchus* all occur in more than 50% of the sites of SG F, but have constancies of only 0.07 in SG G (Table 4.1).

Many of the taxa with high constancies in the forests on sandy soils (SGs D-G), also occur in SG A (dwarf shrubs, grasslands and gaps in forests). The absence or low constancy of the three taxa which were rare in SG G, but which were common in SG F is noteworthy. Taxa highly typical for SG A are *Paratylenchus* A, *Aglenchus*, *Panagrolaimus*, Criconematidae A, *Aporcelaimellus* and cf. *Tripius* (Table 4.1).

Seasonal sampling

The seasonal replicates of the 8 sites which were sampled at three-monthly intervals, as well as the replicates from the site which was sampled tenfold at one occasion, fell into single clusters and were thus classified within the same SGs. Three such sites were assigned to SG A, two each to SG B and E, and one to SG C (Table 4.3). The relative abundance of most taxa varied between the sampling occasions, and taxa occurring in relatively low numbers could often not be detected in some seasonal samples of a site. The presence of each nematode taxon was related to its individual maximum relative abundance. 88% of the abundant taxa (i.e. any taxon reaching 10% in any season at a particular site) were detected in all seasons at their respective localities; if less abundant taxa (5%) are considered, 66% were detected in all seasons.

Thus, although variation in nematode faunal composition due to population dynamics and/or sampling error occurred, the similarity between the samples collected from the same site exceeded those from other, but related, sites.

Environmental characteristics of the Sample Groups

Sample group A (dwarf shrubs, grasslands and gaps in forests) was made up of soils with a sand content >89% and silt content <4.1%. Soil chemical and physical characteristics are comparable to those for SGs F and G, with only pH and nutrient concentrations differing significantly (Table 4.2). According to the criteria defining SGs, SG A could have been subdivided into three separate SGs (grasslands; Scots pine forests with *Deschampsia flexuosa* (L.) Trin. dominating the herb layer; *Calluna* heather,

Table 4.2. Mean physical and chemical characteristics of 0-10 cm mineral soil for each of the seven Sample Groups.

	SAMPLE GROUP						
	A	B	C	D	E	F	G
TEXTURE (%)							
<2μm	2.0 *d*	44.9 *a*	10.6 *b*	9.8 *b*	5.6 *c*	1.8 *de*	1.1 *e*
[2;38>	3.5 *d*	38.1 *a*	23.8 *ab*	22.2 *b*	12.8 *c*	5.1 *de*	1.6 *e*
≥38μm	94.5 *d*	17.0 *a*	65.6 *b*	68.0 *b*	81.5 *c*	88.6 *de*	97.3 *e*
%water	23.7 *cd*	52.9 *a*	35.1 *b*	33.7 *b*	32.9 *bc*	16.2 *d*	15.0 *d*
pF2 (%)	24.3 *c*	53.1 *a*	43.3 *b*	44.5 *ab*	38.6 *b*	8.5 *abc*	24.0 *c*
pF4.2 (%)	7.0 *c*	23.4 *a*	13.5 *b*	15.4 *ab*	10.7 *b*	4.2 *abc*	7.3 *c*
TOC (%)	2.8 *b*	5.6 *a*	4.0 *a*	5.5 *a*	4.5 *a*	2.5 *b*	1.8 *b*
pH(KCl)	5.6 *b*	6.7 *a*	3.8 *c*	3.2 *d*	2.9 *e*	3.2 *d*	3.3 *d*
pH(H_2O)	6.0 *b*	7.3 *a*	4.8 *c*	4.0 *d*	3.7 *e*	4.1 *d*	4.1 *d*
%LIME	0.7 *b*	5.9 *a*	0.0 *b*	0.0 *b*	0.3 *b*	0.0 *b*	0.0 *b*
CEC	7.3 *c*	41.4 *a*	14.1 *b*	15.1 *b*	12.6 *b*	6.4 *c*	5.4 *c*
Base (%)	35.0 *bc*	84.0 *a*	39.4 *b*	9.9 *d*	17.1 *cd*	10.1 *d*	13.5 *cd*
K	0.26 *b*	0.45 *a*	0.27 *b*	0.20 *b*	0.16 *b*	0.08 *c*	0.05 *c*
Ca	18.83 *d*	26.16 *a*	4.05 *d*	1.15 *b*	6.05 *bd*	0.26 *c*	0.51 *bc*
Mg	18.15 *b*	5.10 *a*	0.91 *c*	0.33 *d*	0.31 *de*	0.19 *de*	0.16 *e*
NO_3	-	-	5.1 *ab*	6.0 *a*	5.2 *a*	2.6 *b*	0.7 *b*
NO_3:NH_4	-	-	2.9 *a*	3.2 *a*	1.3 *a*	0.8 *b*	0.2 *b*
N(tot)	-	-	2988 *a*	2810 *a*	1901 *ab*	1237 *b*	339 *c*
C:N	-	-	14.8 *a*	20.4 *b*	22.2 *b*	20.9 *b*	28.7 *b*

%water, soil water content at sampling (fresh weight); TOC, total organic carbon; K, Ca, Mg and CEC (cation exchange capacity) in meqx100 g^{-1}; Base, base saturation; NO_3 and N(tot) in mgxkg^{-1} (dry weight); significant differences ($P<0.05$, Mann-Whitney U-test) are indicated by different letters in the same line.

Corynephoretum dune and gaps in forests where grasses dominated). However, since only 11 sites (23 sampling events) are involved, subdivision was not made.

Only one grassland on sandy soil was not included in SG A, but in SG G. Chemical and physical characteristics of that site differed slightly from those in SG A. However, none of the nematode taxa typical of SG A (Table 4.1) were present.

Sample group B is made up of clayey soils (including one loamy soil) with an average clay content of 44.9% (Table 4.2) and a sand content <33%. Soil water content at sampling, moisture retention at pF2 and pF4.2, concentrations of K, Ca and Mg, CEC and base saturation are significant higher than in other SGs. Due to the presence of lime in six of the nine sites, average soil pH is highest for SG B (pH-KCl=6.7). Both grasslands and forests are present in SG B (Table 4.3), which were not separated in different clusters. The forests are *Salicetum*, *Alnetum* and *Fraxino-Ulmetum typicum* (Table 4.3).

The observed overlap of the SGs C-G in the ordination diagram of the second DCA (Fig. 4.3) also occurs in the chemical, physical and floristic characteristics (Tables 4.2, 4.3 and 4.4). Sample Groups C and D are composed of sites with sandy-loam soils with comparable soil texture, soil water content, pF2, pF4.2, total organic carbon content, CEC and concentrations of nitrogenous compounds and K. Compared to SG D, concentrations of Ca and Mg, Base saturation and soil pH of SG C are significantly higher, whereas the C:N-ratio is lower.

Except for soil texture and pH, the soil chemical and physical characteristics of SG E are very similar to the SGs C and D (Table 4.2). Soil texture of SG E is intermediate to the sandy-loam soils of the SGs C and D and the sandy soils of the SGs F and G. Average bulk soil pH of SG E is the lowest found in this study with pH(KCl)=2.9.

The SGs F and G comprises sandy soils with an average sand fraction >93% and only differ in total-nitrogen concentration, being lower for SG G (Table 4.2). Compared to the other forested SGs (B-E), soil water content, pF, total organic carbon, CEC, NO_3:NH_4-ratio and K, Mg, NO_3 concentrations are lower in the SGs F and G.

The observed gradient in soil chemical and physical characteristics for the SGs B-G, corresponds with changes in the composition of the vegetation of these groups (Table 4.3 and 4.4). *Pinus sylvestris* L. is present in 95% of the sites of SG G, but is rare in the other SGs. Within SG G, 75% of the sites are classified as *Dicrano-pinion*. These forests have a very species-poor herb layer, with only *D. flexuosa* and *Empetrum nigrum* L. present relatively frequently. *Convallario-Quercetum dunense*, *Betulo-Quercetum roboris* and forested former heathlands (i.e. degraded form of *Fago-Quercetum petraeae*) are characteristic forest types of SG F. *Sorbus aucuparia* L., *Lonicera periclimenum* L. and *Vaccinium myrtillus* L. are found frequently in this SG. Sample Group E mainly consists of *Fago-Quercetum petraeae typicum* and *Milio-Fagetum*. The dominant tree species *Fagus sylvatica* L. is present in 95% of the sites, and *Rubus* occurs in 70% of the sites. Sample Group D is a transitional stage between the SGs E-F and C, with on the one hand moist sub-associations of *Fago-Quercetum petraeae* and *Betulo-Quercetum roboris* and on

Table 4.3. Occurrence of vegetation types [1] in the Sample Groups A-G.

VEGETATION TYPE	SAMPLE GROUP						
	A	B	C	D	E	F	G
Cladonio-Pinetum							6
Leucobryo-Pinetum	2 (6)						7
Empetro-Pinetum						1	8
Empetro-Betuletum						1	
Convallario-Betuletum							1
Convallario-Quercetum dunese						9	1
Betulo-Quercetum roboris					1	8	1
Betulo-Quercetum molinitosum				7			
Fago-Quercetum molinitosum				8		1	
Degradated *Fago-Quercetum*				2	2 (16)	9	
Fago-Quercetum petraeae typicum					16 (20)	3	
Milio-Fagetum			2	8	9	6	
Luzulo-Fagetum				4		3	
Stellario-Carpinetum loniceretosum						2	
Stellario-Carpinetum			5 (9)	5		1	
Melico-Fagetum			1		1		
Pruno-Fraxinetum			2				
Fraxino-Ulmetum typicum		1 (5)					
Alninon glutiosae		1			1		
Salicetum		2					
Grassland	4 (8)	4 (8)					1
Others (heathland, forest gaps)	5 (9)				2		3
Total number of sites	11 (23)	8 (16)	10 (14)	34 (34)	32 (50)	44 (44)	28 (28)

1) according to Van der Werf (1991); the number of sites *including* seasonal replicates are given between brackets.

the other hand relatively floristic rich forest types from the *Fagetalia sylvaticae*. Similar to SGs E and F *S. aucuparia, Prunus, Rubus* and to a lesser extend also *Pteridium aquilinum* (L.) Kuhn are found frequently in SG D. The *Fago-Quercetum petraeae molinietosum* and *Betulo-Quercetum roboris molinietosum* sites are typified by the presence of *Molinea caerula* (L.) Moench. The *Stellario-Carpinetum* and *Pruno-Fraxinetum* sites of SG C have a species rich shrub and herb layer with *Anemone*

Table 4.4. Relative frequency of occurrence (0-100%) of a selection of higher plants and ferns in the Sample Groups C-G: forests on sandy-loam to sandy soils.

SPECIES	SAMPLE GROUP				
	C	D	E	F	G
N	6	32	20	43	21
Pinus sylvestris	0	5	0	14	95
Empetrum nigrum	0	0	0	9	43
Rhamnus frangula	0	16	0	14	10
Deschampsia flexuosa	0	13	5	42	52
Crataegus spp.	17	13	0	21	5
Betula spp.	33	34	15	37	33
Fagus sylvatica	50	31	95	44	5
Sorbus aucuparia	17	50	40	60	19
Rubus spp.	17	59	70	40	5
Prunus spp.	17	38	40	33	5
Lonicera periclymenum	33	31	25	40	19
Dryopteris spp.	50	28	10	26	5
Anemone nemorosa	67	9	10	5	0
Oxalis acetosella	67	16	25	9	0
Hedera helix	67	16	40	21	0
Galeobdolon luteum	33	9	5	9	0
Sambucus nigra	33	19	20	16	0
Milium effusum	17	16	25	5	0
Acer pseudoplatanus	17	19	35	23	0
Corylus avellana	33	34	15	14	0
Ilex aquifolium	17	9	30	26	0
Pteridium aquilinum	0	19	25	16	0
Quercus spp.	83	81	75	84	24
Polygonatum multiflorum	0	6	15	12	0
Vaccinium myrtillus	0	16	15	42	0
Molinia caerulea	0	38	0	9	0

N is the number of vegetation descriptions.

nemorosa L., *Hedera helix* L., *Oxalis acetosella* L. and *Dryopteris spec.* frequently found. The forest types present in SG B are unique to this group.

Generally each SG contains several forest types, but most of the forest types are restricted to, or have their optimum of occurrence in, one of the SGs (Table 4.3). However, the forest type *Milio-Fagetum* is dominant in three different SGs.

In conclusion, the results showed that for a range of terrestrial habitats nematode communities can be defined, and these communities can be related to soil characteristics and vegetation. Soil texture, pH and vegetation structure are found to be important discriminating habitat characteristics.

DISCUSSION

Relationships between the composition of the nematode fauna, vegetation and soil type have been studied by a.o. Johnson *et al.* (1972, 1973, 1974), Yeates (1974), Scotto La Massese & Du Merle (1978), Baujard *et al.* (1979), Scotto La Massese & Boulbria (1980) and Bongers *et al.* (1989). In agreement with these studies, in the present study differences in the composition of the nematode fauna appear related to differences in soil characteristics and vegetation type. Soil characteristics seemed to be of dominant importance, as was also noted by Bongers *et al.* (1989) using a different analysis technique. In the present study clayey and coarse sandy soils formed the respective extremes on the gradients extracted by DCA; their nematode fauna differed greatly. However, differences in soil coincided with differences in vegetation, and this will have a marked influence on the distribution of nematodes species.

Especially when soil characteristics were comparable, further classification of the nematode fauna depended on vegetation structure and related differences in e.g. nutrient dynamics and microclimate, as was shown for the sandy soils. Grass and dwarf-shrub habitats were separated from forested sites with comparable soil characteristics. Within the grass and dwarf-shrub habitats, the nematode fauna of the grasslands differed most from those of the forested sites, whereas the heathlands and patches under canopy gaps lay in between.

Inventory studies such as the present work do not permit elucidation of causal relationships between nematodes and biotic or abiotic environmental factors. However, stepwise regression with the sample scores of the DCA as the dependent variable, showed soil texture, pH and water content are best correlated with nematode populations. They explained $>82\%$ of the variation present. In several studies (Johnson *et al.* 1973, Yeates 1968, 1974, Baujard *et al.* 1979, Scotto La Massese & Boulbria 1980) soil water characteristics were found to be of overriding importance for the occurrence of nematodes species. Because of the strong co-correlations between most environmental variables, no

single factor can be selected in the present work. The occurrence of some nematode species, however, probably can be related directly to soil morphological properties. Yeates (1980, 1981) observed correlations between the occurrence of nematode species with relative large labial probolae or cephalic setae and soil macroporosity. Comparable results were found in the present study for *Wilsonema, Cervidellus, Teratocephalus* and *Metateratocephalus*. These correlations are thought to be related to locomotion and/or feeding requirements of the nematode species (Yeates 1981, De Ley 1991). Except for root-feeding nematodes, causal relationships between occurrence and habitat are unclear for most other species.

Based on the composition of the nematode fauna, soils can be classified into clusters (SGs) which can also be described by combinations of soil physical, chemical and floristic properties. As in many classifications, transitions between clusters were gradual, and this hampers classification of specific samples, especially for samples similar to SGs D-G (forests on sandy-loam to sandy soils). The apparent clear distinction between SGs A, B, C and D-G, probably reflects the selection of only a few SG A-, B- and C-sites (11, 8 and 11 sites respectively), as opposed to 138 sites for the SGs D-G. Extending the number of forested clayey and loamy soils and grassland sites in the classification, will probably blur clear distinctions. Moreover, such an intensified sampling could also result in a further sub-division of these SGs.

Nevertheless, taking into account the relatively large differences in soil properties and vegetation type present in the SGs D-G, the high similarity of the nematode fauna of those SGs is remarkable. This agrees with the findings of Johnson *et al.* (1972) who found, despite differences in pedology and forest type, high similarities (>60%) between 15 of the 18 forests studied. Wasilewska (1970) assumed that poor natural habitats on sandy soils in Northern and Central Europe are characterized by similar nematode species. Such high similarities between geographically isolated sites with similar vegetation and edaphic factors were found also by Norton & Hoffmann (1974) for plant parasitic nematodes in USA.

For the Dutch situation the high similarity between the nematode faunae of different vegetation types on sandy soils, possibly indicates a general trend in the nematode community, related to the trend of decreasing pH in this series of soils. These forested sandy soils had very low pHs, with SG E having an average pH(KCl) of even less than 3.0 (Table 4.2). In Chapter 3, using the Maturity Index technique (Bongers 1990b), it was shown that artificial acidification of forest soils resulted in a decrease of nematode taxa belonging to c-p groups 3-5 (K-strategists *sensu latu*, relatively sensitive to environmental disturbances). Compared to the other SG's, taxa belonging to c-p groups 3-5 were scarce in many sites in SG E (Fig. 2.3). The nematode fauna of SG E was

dominated by taxa belonging to c-p groups 2 which are thought to be relatively stress tolerant (Chapter 2). Moreover, in the samples of SGs C-G there was a significant ($P \leq 0.01$) positive correlation between pH and the proportion of c-p 3-5 taxa, and in addition SG E was the only SG in which fungal feeding nematodes were more abundant than bacterial feeding nematodes.

One of the applications of ecological soil classification systems is the assessment of the condition of soils. Although only nature reserves or lightly managed soils were studied, examples of the potential of the nematode fauna composition as an indicator can be demonstrated. In Scots pine forests, the nematode fauna appeared to indicate that the vegetation of certain sites was not the natural vegetation. This follows observations by Jordana *et al.* (1987), who found significant differences in nematode fauna structure between natural and exotic vegetation on comparable soil types. In the present study, most Scots pine forests were classified in SG G. However, two sites (Hernense Bos and Wageningen forest) were placed in SG A, being plotted between heathlands and grass-covered gaps in forests on the one hand and grasslands on the other hand. The actual vegetation of both forests (Scots pine forest with a herb layer dominated by *Deschampsia flexuosa*) was comparable to several forests within SG G. However, the soil characteristics of both forests were inconsistent with what is expected for a *Dicrano-Pinion* (Van der Werf 1991). The natural vegetation of Wageningen forest is probably a degraded form of *Fago Quercetum petraeae* (Van der Werf, pers. comm.). Thus, although the nematode fauna of both sites possessed characteristics of the *Dicrano-Pinion* (*Cephalenchus* and *Criconematidae C* absent, *Plectus* A present), the presence of nematodes typical of grasslands (*Paratylenchus* A, *Helicotylenchus*) and dwarf-shrub vegetation (*Aglenchus*) indicate inconsistencies between actual and expected natural vegetation on these soils. At Hernense Bos many plant parasitic nematode taxa characteristic to SG A (*Paratylenchus* A, *Aglenchus, Pratylenchus, Helicotylenchus* and *Hemicriconemoides*) were found. It is not inconceivable that this is related to the low vitality of the stand.

Effects of seasonal fluctuations on the nematode community in soil classification studies are scarce. Jordana *et al.* (1987), who compared soil fauna populations of different vegetation on comparable soil types, showed that seasonal fluctuations in the composition of the nematode fauna were minor to differences due to vegetation type. Similar findings were reported by Yeates (1984), who concluded that site was more important than month in determining population composition of grazed pastures. In our study differences in nematode fauna structure due to seasonal fluctuations and techniques used, also appeared to be relatively small compared to differences in nematode fauna

structure between different sites. Of course it can be argued that this supposed season-independency may have resulted from selecting relatively extreme sites for repeated sampling. However, the repeatedly sampled sites, especially those within the SGs C and E, had vegetation and soil characteristics similar to other sites of these SGs, but classified in different clusters. Moreover, the samples taken in autumn 1985 and spring 1988 are intermixed within the SGs and clusters. Thus time of sampling does not affect the classification of sites.

The assessment of the nematode fauna was restricted to the collection of one bulk soil sample of the 0-10 cm mineral soil collected from 100 m^2. Because of the impracticability of homogenizing organic and mineral horizons and because of large differences in methodology for optimal nematode extraction from such horizons (Schouten *et al.* 1990, Schouten & Arp 1991) inclusion of both the organic horizons and the mineral soil in one bulk sample was avoided. The 0-10 cm mineral soil was selected, because in contrast to the organic horizon it is present under most vegetation types found in the Netherlands. Only some peat soils lack a mineral horizon, or when present, this horizon could not be reached. Therefore, these soils were excluded from this study.

Restricted sampling of only 0-10 cm mineral soil, however, resulted in the exclusion of the principal part of the nematode fauna of soils with a mor or moder humus (most soils of SGs D-G). This has to be considered when using the results of this study to compare ecological implications of the different SGs. The nematode distribution in the soil profile largely depends on the distribution of organic material (Yeates & Coleman 1982). In mor and moder humus, highest nematode abundances were found in the fermentation horizon of the organic layer (Popovici 1980, Chapter 5), whereas in mull humus highest numbers occurred in the upper centimetres of the mineral soil (Popovici, 1980).

The exclusion of the organic horizon will, as was shown in this study, probably not be inconvenient in a habitat classification to assess groups of soils with similar ecological requirements. Also Arpin & Ponge (1986) showed that the nematode fauna of mineral soil horizons possessed discriminating abilities between habitats. They compared the composition of the nematode fauna of different soil horizons of a Scots pine (*P. sylvestris*), an oak (*Quercus petraea* (Mattuschka) Lieblein) and a mixed pine-oak forest. Differences between these forests were best revealed by differences in nematode fauna structure of the deeper soil layers, corresponding to the 6-10 cm mineral soil of the Scots pine forests. It should be noticed however, that the similarity between corresponding horizons of the different forests studied by Arpin & Ponge (1986) appeared to be larger than the similarity among the different horizons within each forest. Similar results were obtained in Chapter 5, where nematological changes during primary succession of Scots

pine forest were studied. Inclusion of the nematode fauna of these organic horizons may contribute to a more refined classification of the SGs D-G.

The abundance distribution of nematode species in soil samples is usually skewed, with a majority of species being relatively rare. Several such relatively rare species (e.g. those in the genera *Boleodorus, Pungentus, Coslenchus, Basiria* and *Diphtherophora*) (Bongers *et al.* 1989), appeared important to the classification. However, as the detection level of a species depends on the relative abundance of the species, the position of relatively rare species in a classification needs special attention. Besides, many nematodes show high dispersion abilities (Proctor 1984), and can therefore be found also outside their optimum home range. *Wilsonema*, a genus restricted to sandy soils, was found in one of the grasslands with a clayey soil, and Criconematidae were reported from submerged sediments in an oil harbour (Tamis 1986b). The assessment of single samples will inevitably be biased by such chance observations.

The classification described in this study was based on all nematode taxa occurring in the samples. However, recent studies (Ettema & Bongers 1993, Zell 1989) indicated that the abundance of species within the Rhabditidae in some situations depends on brief periods of increased food availability, rather than habitat structure. Similar relationships can be expected also for species of Diplogasteridae and Panagrolaimidae which also belong to c-p group 1 (Bongers 1990b). During such periods of increased food availability these taxa can make up >50% of the total nematode fauna. Extreme dominance of these species in soil samples can also be the result of accidental occurrence (e.g. a dropping of a rabbit or a small animal corpse in a core). The insect parasitic genus *Steinernema*, the juveniles of which were found frequently in both grasslands and forests on sandy soils also shows pockets of abundance; in infected insects numbers in excess of 800,000 individuals of *Steinernema* have been recorded (Poinar 1983). Although nematodes belonging to c-p group 1 have important potentials as indicators of environmental conditions (Bongers 1990b, Ettema & Bongers 1993), their value to an ecological soil classification is debatable.

In conclusion the nematode fauna can contribute to an ecological soil classification, which can serve as a reference to a biological soil assessment system. Moreover, analysis of the nematode community structure itself, either by direct interpretation or by evaluation of derived indices, offer prospects of assessing the ecological condition of soils.

ACKNOWLEDGEMENTS

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CHANGES IN NEMATODE COMMUNITY STRUCTURE IN A PRIMARY SUCCESSION OF BLOWN-OUT AREAS IN A DRIFT SAND LANDSCAPE

SUMMARY

Nematode community structure and nematode abundance in a primary succession of blown-out areas in a drift sand landscape were studied. The successional stages sampled included drift sands without vegetation, Spergulo-Corynephoretum *and Scots pine (*Pinus sylvestris *L.) forests of different age. Samples were taken from the 0-10 cm mineral soil and, in the forested stages, also from the organic layer. In order to study succession of the nematode fauna in relation to soil development, the organic layer was divided into a litter, fermentation and humus horizons. Multivariate analyses showed gradual changes in nematode faunal structure as succession proceeded. The colonization of the sites by higher plants, and the subsequent invasion of Scots pine coincided with marked differences in the composition of the nematode fauna. The diversity of the nematode fauna increased as soil development proceeded. Differences in nematode fauna structure between the soil horizons within any successional stage appeared larger than differences between the successional stages of the soil horizons. The successional changes in the composition of the nematode fauna are discussed in terms of the colonizers-persisters model of Bongers, and the prospect for nematodes to contribute to a soil classification system are indicated.*

INTRODUCTION

Studies on primary forest succession concentrate mainly on the development of the vegetation (West *et al.* 1981). Below-ground processes are usually included in terms of horizon development, and root and nutrient dynamics. While studies on changes in soil faunal population structure in relation to primary forest succession are rare (Heal & Dighton 1986), extensive information is available about the occurrence and functioning of the soil fauna of various habitats (Swift *et al.* 1979, Petersen & Luxton 1982, Mitchell & Nakas 1986).

Studies of short-term successional changes in soil fauna populations on freshly fallen leaves or during the colonization of litterbags (e.g. Twinn 1974, Anderson 1975, Sohlenius & Boström 1984, Zell 1989, Siepel 1990) and further analyses of later stages of decomposition can reveal a picture of soil faunal changes during natural succession within various ecosystems (Twinn 1974, Rusek 1978, MacMahon 1981). However, despite their usefulness, trends deduced will often be crude due to climatological, geological, pedological and methodological differences between different studies. Fine-scale

investigations of successional changes in soil faunal populations, either derived from long-term sampling programmes or from studying successional seres, are necessary to test the significance of the suggested patterns of faunal succession (e.g. Odum 1969, Rusek 1978, Heal & Dighton 1986) under field conditions. In addition, with growing interest in soil faunal community structure as an indicator of environmental disturbance, knowledge concerning both short-term and long-term natural changes in community structure, become a prerequisite to identify such disturbance.

We studied changes in nematode faunal composition in relation to primary vegetation succession of blown-out areas in a drift sand landscape in the Netherlands. Nematodes are among the soil faunal groups which offer possibilities for assessing soil ecosystem quality (Samoiloff 1987, Freckman 1988, Bongers 1990b), and are potentially useful in ecological soil classifications (Bongers *et al.* 1989). Drift sands are among the most nutrient poor soils in North-Western Europe. *Cladonio-Pinetum* (Juraszek 1927) as the first forested stage of drift sands is consequently highly sensitive to stresses such as those which may be imposed by atmospheric deposition (Van der Werf 1991). In view of the relatively high levels of deposition of especially nitrogenous compounds in the Netherlands (Heij & Schneider 1991), knowledge of the natural soil community and its succession within these habitats is urgently needed. The blown-out areas at 'Hulshorst sand' and 'Leuvenum forest' offer possibilities to sample across a vegetation succession of the drift sand habitat. Past forest development of the area is well documented, prior soil development of the blown-out soils is lacking, and the texture and mineralogical composition of the parent material of the soil remains more or less constant during the first centuries of succession (Fanta 1986, Emmer *et al.* 1991). Moreover, succession of vegetation (Fanta 1986, Prach 1989), its nutrient dynamics (Moszynska 1991) and soil development (Van Berghem *et al.* 1986, Emmer *et al.* 1991) are well documented. However, as this area is subjected to atmospheric deposition, the influence of this deposition on the biological succession, as well as the influence of changing climatic conditions on the succession, cannot be excluded.

This paper presents details on nematode community structure and nematode abundance at blown-out areas at Hulshorter sand and Leuvenum forest. Distribution of nematode taxa within the soil profile, trophic group distribution and nematode biomass in various successional stages are given in Chapter 6.

MATERIALS AND METHODS

Site description

The study was undertaken in 'Hulshorst sand' and 'Leuvenum forest', two adjacent nature reserves near Harderwijk in the Netherlands. Descriptions of the geology, pedology and vegetation of the area are given in Prach (1989), Van Berghem *et al.* (1986) Emmer *et al.* (1991) and Fanta (1986).

The area is a former cover sand landscape which was covered with forests and heathlands. Mainly due to overgrazing, the sand became active, and the area was converted into a drift sand landscape. However, since the first half of the 19th century the drift sands were reforested by both spontaneous establishment and planting (Prach 1989). Our research was conducted in blown-out areas. Their substrate is a gravel-rich, coarse fluvio-periglacial sand, with a very low organic content (0.2-0.4% by weight) (Emmer *et al.* 1991).

The first stages of primary succession are characterized by the occurrence of algae, lichens, and low growing plants such as *Corynephorus canescens* (L.) Beauv., *Spergula morisonii* Bor. and *Polytrichum piliferum* Hedw. (Prach 1989, Fanta 1986). The subsequent invasion of Scots pine (*Pinus sylvestris* L.) coincides with the occurrence of *Festuca ovina* L.. At the same time an organic horizon starts to develop. With increasing thickness of the organic horizon, *Deschampsia flexuosa* (L.) Trin. becomes the dominant species in the herb layer. After the forest reaches an age of about 90-100 years, *Empetrum nigrum* L., *Vaccinium vitis idaea* L., *Vaccinium myrtillus* L., as well as second generation Scots pine, start to develop. A Scots pine forest about 105 years old (Stille Eenzaamheid, located in 'Leuvenum forest'), characterized by a herb layer composed of *D. flexuosa*, *E. nigrum*, *V. vitis idaea*, *V. myrtillus* forms the oldest stage of succession present. This stage is considered to be a disclimax, because broadleaved tree and shrub species characteristic of the next successional stages (*Betulo-Quercetum*), cannot regenerate successfully due to animal browsing (Fanta 1986). *Deschampsia flexuosa* is still the dominant herb species, but because the density of flowering shoots is low and the density of dead roots is relatively high, its vitality seems to be reduced (Moszynska 1991).

Soil development took place, reflecting succession of the vegetation, with mull and mor type humus in the unforested and forested stages respectively. So far, during succession soil texture and mineralogic composition of the parent material of the soil remained fairly constant, but some chemical properties, like pH and amount of organic matter, changed (Emmer *et al.* 1991).

Sampling, extraction, counting and identification

Nematode samples were taken in two spontaneous primary succession seres (sere A and B), with Scots pine forests of about 105 years old as the oldest stage studied.

Sere A consisted of six vegetation successional stages: (1) bare drift sand; (2) *Spergulo-Corynephoretum*; Scots pine forests of (3) 5-8, (4) 30-35, (5) 80 and (6) 105 years old. Stages 1-5 were located at 'Hulshorst sand', whereas the oldest stage (stage 6) was located 2.3 km away at De Stille Eenzaamheid.

Samples were taken on 24 and 25 October 1988. Within each stage, three representative 100 m^2 plots were selected. The organic horizons and mineral soil were sampled separately.

From the organic horizons ten cores (core diameter 79 mm) were taken, and combined into one bulk-sample per plot. From stage 4 onwards organic horizons covered the whole soil surface. In stages 1 and 2 no organic horizon was present. In stage 3 the organic horizon was present only under the canopy of the scattered, young Scots pine trees within each plot. Therefore, the organic horizon of stage 3 was sampled specifically under the canopy of those trees.

The mineral soil was sampled to a depth of 10 cm. Fifty cores (core diameter 17 mm) were taken in a regular pattern over the whole plot, and combined into one bulk-sample per plot.

Sere B consisted of seven successional stages and an eighth stage which was thought to be an example of an early variant of *Betulo-Quercetum* (forest composed of *P. sylvestris, Quercus robur* L. and *Betula* spec., with a herb layer dominated by *E. nigrum* or *V. myrtillus*; *D. flexuosa* covered $\leq 5\%$ of the area of these plots). The first six stages were located at 'Hulshorst sand' along a transect with a length of 950 m, about 500 m south of sere A. Stage 7 was located 2.6 km away at De Stille Eenzaamheid, and stage 8 was located 1 km north-east of stage 7 at 'Leuvenum forest'. The stages sampled can be characterized as: (1) bare drift sand; (2) *Spergulo-Corynephoretum*; Scots pine forests of (3) 3-5, (4) 25-30, (5) 45-50, (6) 80-90 and (7) 105 years old; (8) *Betulo-Quercetum* of approximately 105 years old.

Within every stage, three (four in stages 2 and 8) representative 100 m^2 plots were selected. The mineral soil and organic horizons (stage 3-8) were sampled separately on 11 and 12 June 1990. The organic layer was divided into litter (L), fermentation (F) and humus (H) horizons following Klinka *et al.* (1981).

The samples of the organic horizons were composed of one sample per plot only, and were taken 1.5 m from a tree trunk. The L-horizon, present from stage 3 onwards, was sampled by hand from 900 cm^2. The F- and H-horizons, present respectively from stages

Table 5.1. Mean nematode abundance and total number of genera (n=38) in the 0-10 cm mineral soil (M) and organic horizon (S) of the successional stages of sere A.

SUCCESSIONAL STAGE	NEMATODE ABUNDANCE		NUMBER OF TAXA	
	M[1)]	S[2)]	M	S
1	49 *a*	-	13	-
2	721 *b*	-	17	-
3	942 *b*	1186	17	8
4	910 *b*	12690	24	18
5	399 *c*	12070	17	18
6	204 *c*	15460	18	17
TOTAL			33	25

1) numbers per gdwt; 2) numbers $x10^3$ per m^2; Abundances within one column followed by a different letter differ significantly, $p \leq 0.05$; - organic horizon not present.

Table 5.2. Mean nematode abundance (numbers $x10^3$ per m^2) and total number of genera (n=56) in different soil horizons of the successional stages of sere B.

SUCCESSIONAL STAGE	NEMATODE ABUNDANCE				NUMBER OF TAXA					
	M	H	F	L	M	H	F	L	O	T
1	56 *a*	-	-	-	4	-	-	-	-	4
2	461 *bce*	-	-	-	26	-	-	-	-	26
3	485 *bce*	-	-	240 *ab*	21	-	-	9	9	25
4	363 *bcde*	-	539 *a*	196 *ad*	20	-	9	6	11	22
5	412 *b*	264 *a*	1136 *b*	427 *b*	25	12	13	8	20	33
6	824 *c*	476 *b*	1940 *bc*	261 *bd*	22	14	15	8	21	29
7	156 *d*	284 *ab*	2339 *c*	150 *adc*	20	14	18	6	22	28
8	247 *e*	763 *ab*	5000 *c*	68 *c*	33	20	19	10	28	37
TOTAL NUMBER OF DIFFERENT TAXA					51	26	21	16	35	56

0-10 cm mineral soil (M), humus (H), fermentation (F), litter (L) horizon, total organic horizon (O) and total profile (T=M+O); abundances within one column followed by a different letter differ significantly, $p \leq 0.05$; - horizon not present.

4 and 5 onwards, were sampled with a Shallow Profile Sampler (internal surface area 36.5 cm^2; Wardenaar 1987).

The mineral soil was sampled as described for sere A. In the stages 3 and 4 samples were taken specifically under the canopy of the scattered trees and each bulk-sample consisted of fifty cores, evenly distributed under 5 and 2 different trees respectively.

Nematode extraction, counting, processing and identification followed Chapter 3. Nematodes were extracted from 100 g fresh mass mineral soil, 25 g fresh mass organic horizon (sere A), 10 g fresh mass L-horizon and 20 g fresh mass F- and H-horizon. Extractions were completed within two days of sampling. Using known total sample weight, results were converted to a m^{-2} basis.

Water content of each sample was determined after 24 h drying at 105°C.

Statistics

Changes in population structure following succession were analyzed using correspondence analysis (CA) (Ter Braak 1988). The analyses were based on relative generic abundances, which were transformed as follows: 2^n% becomes (n+1)%, when n=0-7. Genera with a frequency less than 20% of the commonest taxon, were downweighted in proportion to their frequency (Ter Braak 1988).

Similarities between samples were calculated with the Similarity ratio (SR) (e.g. Jongman *et al.* 1987); $SR_{ij} = \Sigma_k Y_{ki} Y_{kj} / (\Sigma_k Y^2_{kj} + \Sigma_k Y^2_{kj} - \Sigma_k Y_{ki} Y_{kj})$ with Y_{ki} and Y_{kj} being the proportion of taxon Y in sample i and j respectively. Prior to calculation, data were transformed as described above.

To calculate the c-p value distribution (Chapter 2) of the nematode samples, the c-p scores as given by Bongers (1990b) were used.

Differences in nematode abundance and similarity between the successional stages were tested by Mann-Whitney U-test (Sokal & Rolf 1981).

RESULTS

The correspondence analyses gave clearly differing groups which could be related to the stage of succession and soil horizons. The nematode faunal composition of bare drift sand (M1), differed greatly from the subsequent stages where vegetation was present (Fig. 5.1 for sere A; data for sere B are not shown, but were comparable to Fig. 5.1). In bare drift sand, relatively low numbers of nematodes occurred (Tables 5.1, 5.2) and compared to the mineral soil of the subsequent successional stages, only fewer nematode taxa were

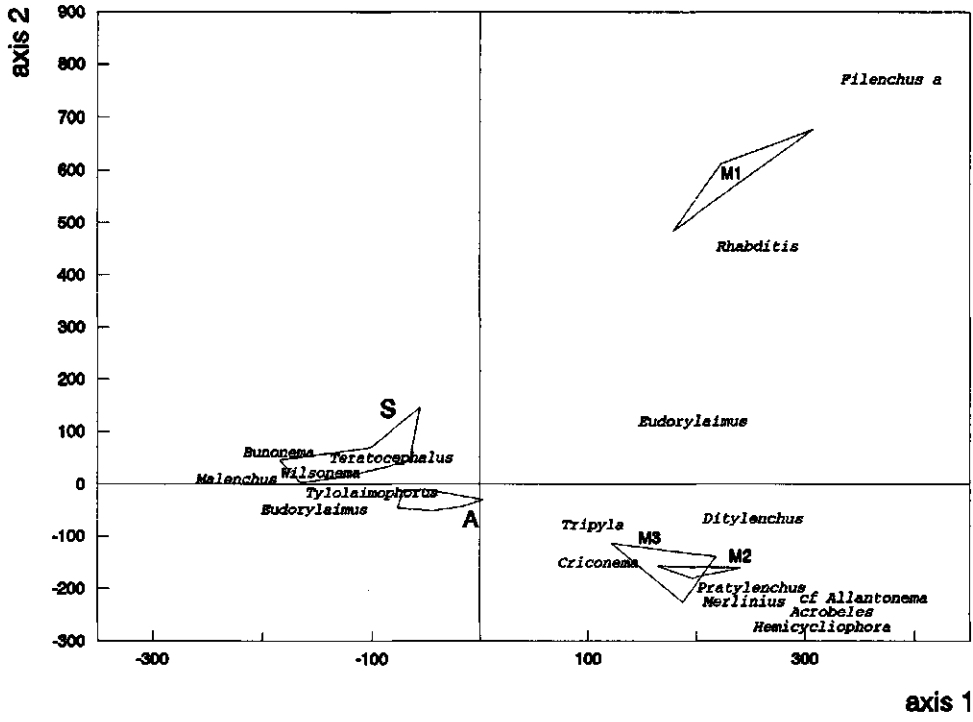

Figure 5.1. Ordination diagram (CA) of the axes 1 and 2 based on the nematode fauna of the organic horizons and 0-10 cm mineral soil of primary succession sere A. Contour lines indicate the position of the samples of the organic horizons (S) and of the 0-10 cm mineral soil of stages 1, 2, 3 and 4-6 (M1, M2, M3 and A respectively). Some common taxa are indicated (each name is centered at the position of the taxon in the diagram).

found (Tables 5.1, 5.2). *Eudorylaimus* was present in all samples from stage 1, and was the dominant taxon. In sere A *Filenchus a* and *Rhabditis* were also found in all three replicate stage 1 samples, but in relatively low numbers.

Based on the composition of the nematode fauna the stages with vegetation present, were divided in two groups differing in stage of succession (stages 2-3 and stages 4-9; Figs 5.1, 5.3). This division was most evident in the composition of the nematode fauna of the mineral soil, but appeared also for the litter horizon of stage 3 (Figs 5.2, 5.3). In both successional seres *Hemicycliophora, Pratylenchus, Acrobeles* and *Merlinius* were characteristic for stages 2 and 3, whereas *Wilsonema, Tylolaimophorus, Filenchus b, Teratocephalus* and *Steinernema* were absent or rare in the early stages but common in the older stages of succession. Moreover, the taxa *Ditylenchus* and cf *Allantonema* and the genera *Zeldia, Aporcelaimellus* and *Paraphelenchus* were restricted to these early stages of the successional seres A and B respectively.

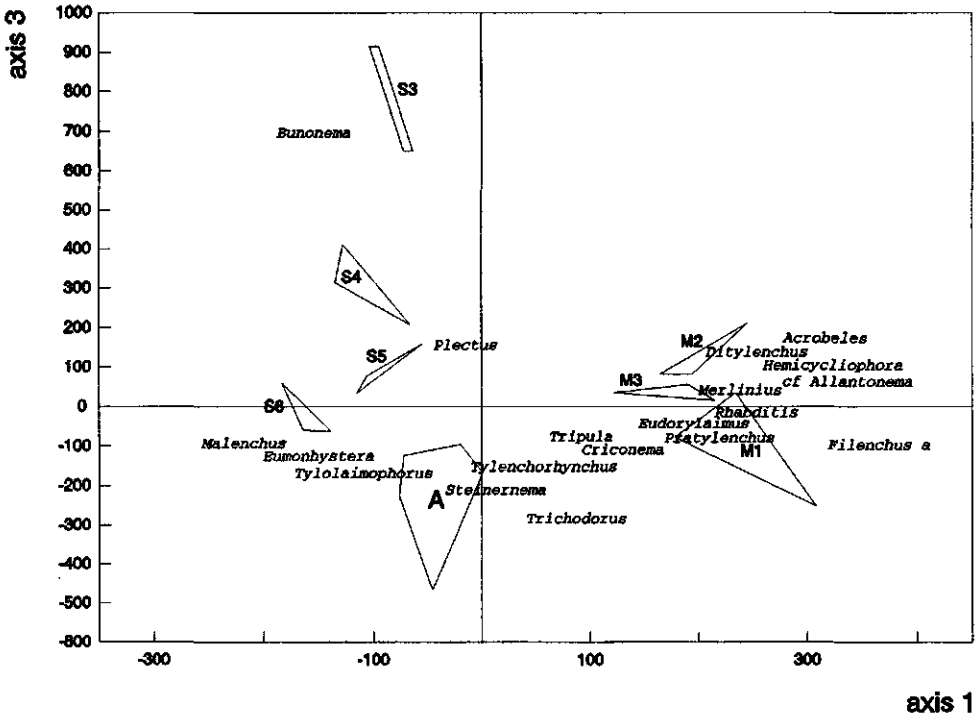

Figure 5.2. As Fig. 5.1, but for the axes 1 and 3 of succession sere A. Contour lines indicate the position of the samples of the organic horizons of the stages 3 to 6 (S3-6) and of the 0-10 cm mineral soil of the stages 1, 2, 3 and 4-6 (M1, M2, M3 and A respectively). Some common taxa are indicated (see Fig. 5.1).

Independent of the stage of succession, a further sub-division of the forested sites (stage 4-9) was related primarily with soil horizon (Figs 5.1, 5.3), which itself is part of succession. In sere B, where the most detailed sub-division in soil horizons was carried out, analysis of the composition of the nematode fauna revealed three groups of soil horizons: litter horizon, fermentation-humus horizon, and 0-10 cm mineral soil. However, subsequent correspondence analyses showed gradual shifts in nematode fauna structure within the horizons, which were related to the stage of succession of the vegetation (Figs 5.3, 5.4, 5.5).

The litter horizon was distinguished from the underlying horizons by the low number of taxa (Table 5.2) and by the occurrence of *Panagrolaimus, Laimaphelenchus* and *Deladenus*. The first two taxa were abundant also in the 0-10 cm mineral soil of the stages 2 and/or 3, which explained the joint classification of the 0-10 cm mineral soil of the stages 2-3 and the L horizons on the first CA axis (Fig. 5.3), despite a relative low similarity between litter horizons and 0-10 cm mineral soil (SR 25-42%).

Differences in nematode fauna composition between the F/H horizon and the 0-10 cm

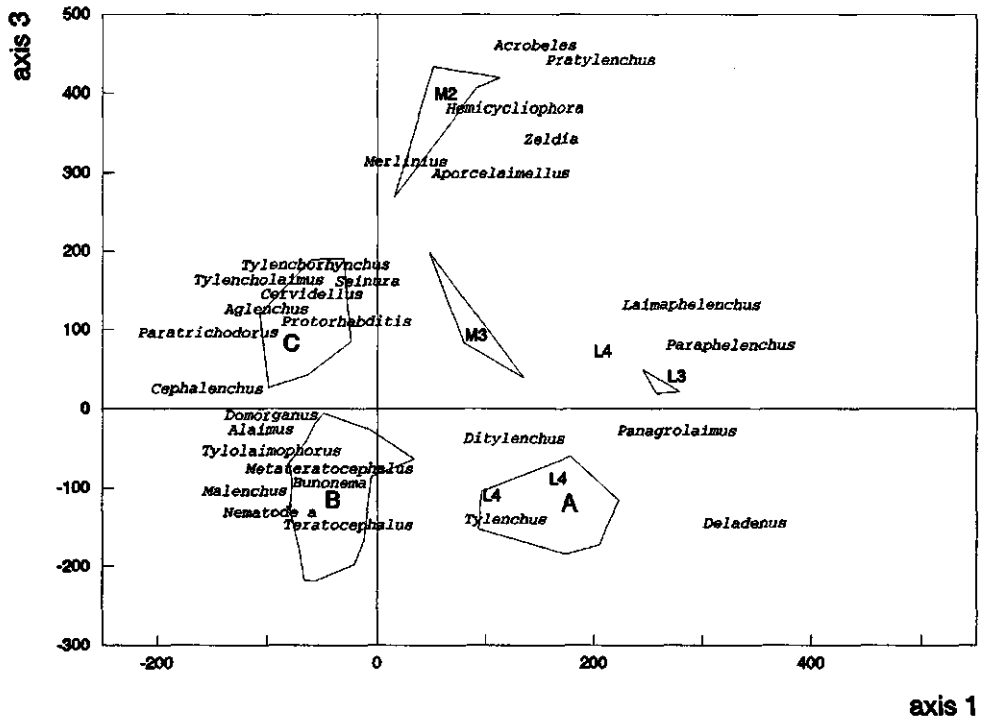

Figure 5.3. Ordination diagram (CA) of the axes 1 and 3 based on the nematode fauna of the organic horizons and 0-10 cm mineral soil of primary succession sere B. Contour lines indicate the postition of the samples of the litter, fermentation/humus and 0-10 cm mineral horizons of stages 4-8 (A, B and C respectively), of the 0-10 cm mineral soil of stages 2 and 3 (M2, M3) and of the litter horizon of stages 3 (L3) and 4 (L4). The pattern extracted by the axis 2 is not shown, but was comparable to the gradient extracted by axis 2 of the CA based on the nematode fauna of sere A. Some common taxa are indicated (see Fig. 5.1).

mineral soil were primarily based on *Cervidellus* (respective frequencies (%) in 0-10 cm mineral soil and F/H horizon 100, 6; n=16), *Tylenchorhynchus* (100, 12), *Tylencholaimus* (50, 0), *Aglenchus* (44, 6) and *Paratrichodorus* (38, 0), which were characteristic for the 0-10 cm mineral soil, and *Teratocephalus* (6, 56) and Nematode A (6, 50; an undescribed alaimid) which occurred more frequently in the F/H horizon.

Nematode diversity was highest in the 0-10 cm mineral soil where 33 and 51 different taxa were found in seres A and B respectively (Tables 5.1, 5.2). In the organic horizon of seres A and B, 25 and 35 taxa were detected respectively. Exclusion of the successional stages without an organic horizon (stages 1-2) and/or those with only a litter horizon present (stage 3), only partly explained this higher diversity in the 0-10 cm mineral soil; the total number of taxa found in the 0-10 cm mineral soil of the stages ≥ 3

and ≥4, was 30 and 26 for sere A and 48 and 43 for sere B respectively. Except for an increase in nematode taxa related to the development of vegetation (stage 2), another remarkable increase in nematode taxa occurred at the change from stage 4 to 5 of sere B (Table 5.2). This increase was brought about mainly because of the development of the organic horizon, as genera increased in the organic horizon and 0-10 cm mineral soil by 82% and 25% respectively. In the subsequent successional stages total numbers of genera remained fairly constant, but shifts in the composition of the nematode fauna still occurred (Figs 5.4, 5.5).

The relation between successional stages and the composition of the nematode fauna of the various horizons of sere B are summarized in Table 5.3, where the similarity between stage 7 and the other successional stages is given for each horizon. The similarity between the stages 1-6 and stage 7 increased with proceeding succession, whereas a subsequent decrease was found for stage 8. The relatively large increase in similarity when going from stage 3 to stage 4, is in line with the results from the correspondence analysis (Fig. 5.3). Thus, the development of an organic horizon concurs with major changes in the composition of the nematode fauna, even in the 0-10 cm mineral soil.

In the field seral stages 3 and 4 form a patchwork surrounded by stage 2 vegetation. Nematode species occurring in stages 3 or 4 and which do not occur in stage 2, have to colonize these stages. This colonization can take place from *i)* nearby stage 3 and 4 patches, or from *ii)* the older forested stages (stage ≥5). Of the 19 taxa which had to colonize the stages 3-4 (Table 5.4), *Laimaphelenchus penardi*, *Acrobeloides tricornis* and *Aporceliamium* occurred only in the stages 3-4, and are probably examples of type i) colonization. Compared to the taxa occurring both in stage 2 and in the stages ≥3-4, a

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Legend Figure 5.4. Ordination diagram (CA) of the axes 1 and 2 based on the nematode fauna of the organic horizons of primary succession sere B. Contour lines indicate the position of the samples of the litter horizons of the stages 3 and 4-8 (L3 respectively A) and of the fermentation (F) and humus (H) horizons of the stages 4-8 (F4-8, L4-8). Some common taxa are indicated (see Fig. 5.1).

Legend Figure 5.5. Ordination diagram (CA) of the axes 1 and 4 based on the nematode fauna of the 0-10 cm mineral soil of primary succession sere B. The positions of the samples of the stages 1-8 are indicated with close fitting contour lines (M1-8). The patterns extracted by the axes 2 and 3 are not shown, but were similar to those extracted by the axes 2 and 3 respectively of the CA based on both organic and mineral soil horizons. Some common taxa are indicated (see Fig. 5.1).

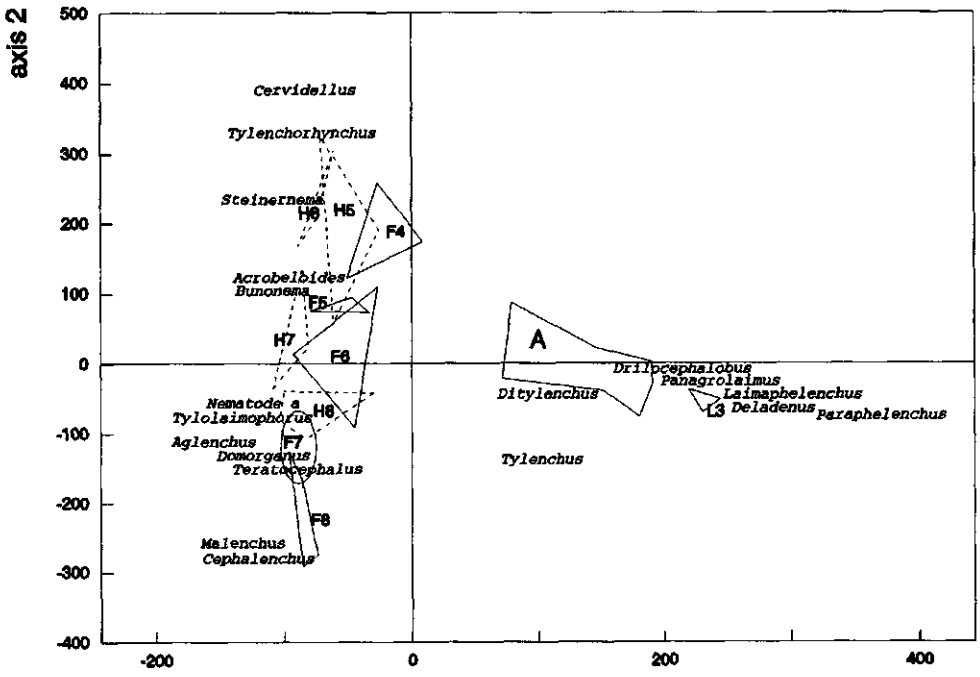


Figure 5.4. Legend at opposite page.

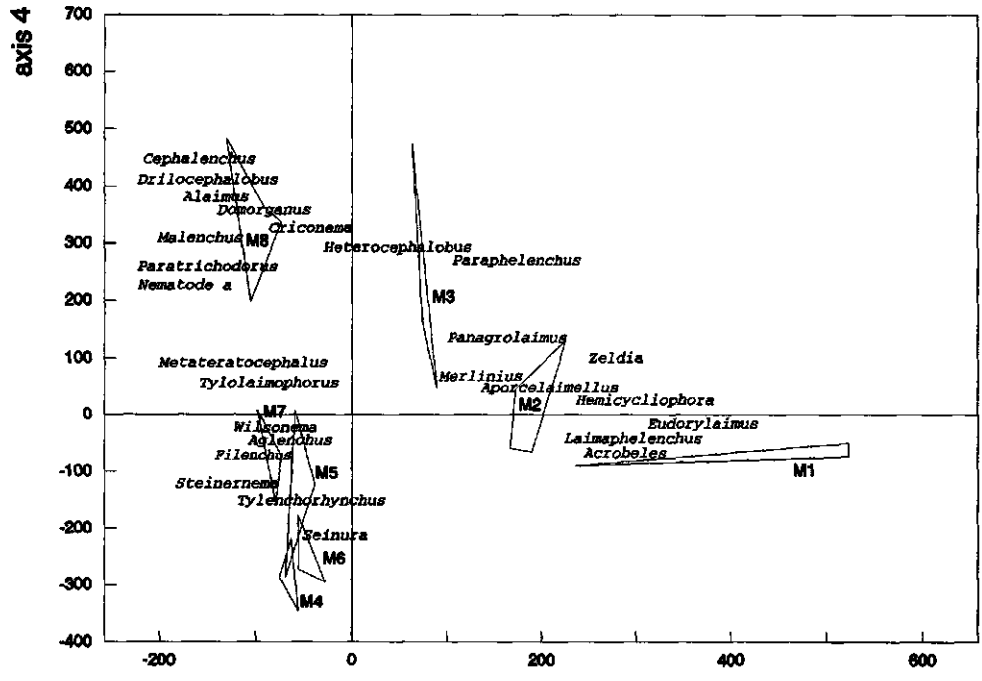


Figure 5.5. Legend at opposite page.

Table 5.3. Similarity (SR) between the nematode fauna of successional stage 7[1)] and the other stages of sere B by horizon.

SUCCESSIONAL STAGE	SOIL HORIZON M		H		F		L	
1	5.8	*a*	-		-		-	
2	29.1	*b*	-		-		-	
3	33.4	*b*	-		-		56.1	*a*
4	66.7	cd	-		53.4	*a*	65.5	*ad*
5	79.0	*e*	68.1		73.1	*b*	82.2	*bc*
6	70.9	*c*	68.2		70.9	*b*	85.6	*c*
7	(83.2)		(78.4)		(84.8)		(90.0)	
8	64.9	*d*	64.5		75.9	*b*	75.8	*bd*

1) For stage 7 the internal-similarity between replicates is given; abbreviations as in Table 5.2; similarities within one column followed by a different letter differ significantly, $p \leq 0.05$, - combination not present.

Table 5.4. Number of nematode taxa (n=39, excluding the plant feeding taxa) of successional sere B tabulated by c-p group and distribution within successional sere.

C-P GROUP	TAXA PRESENT IN THE STAGES ≥2	≥3-4	TAXA PRESENT ONLY IN STAGES 5-8
1	0	3	0
2	6	12	2
3	2	3	2
4	2	0	2
5	1	1	0

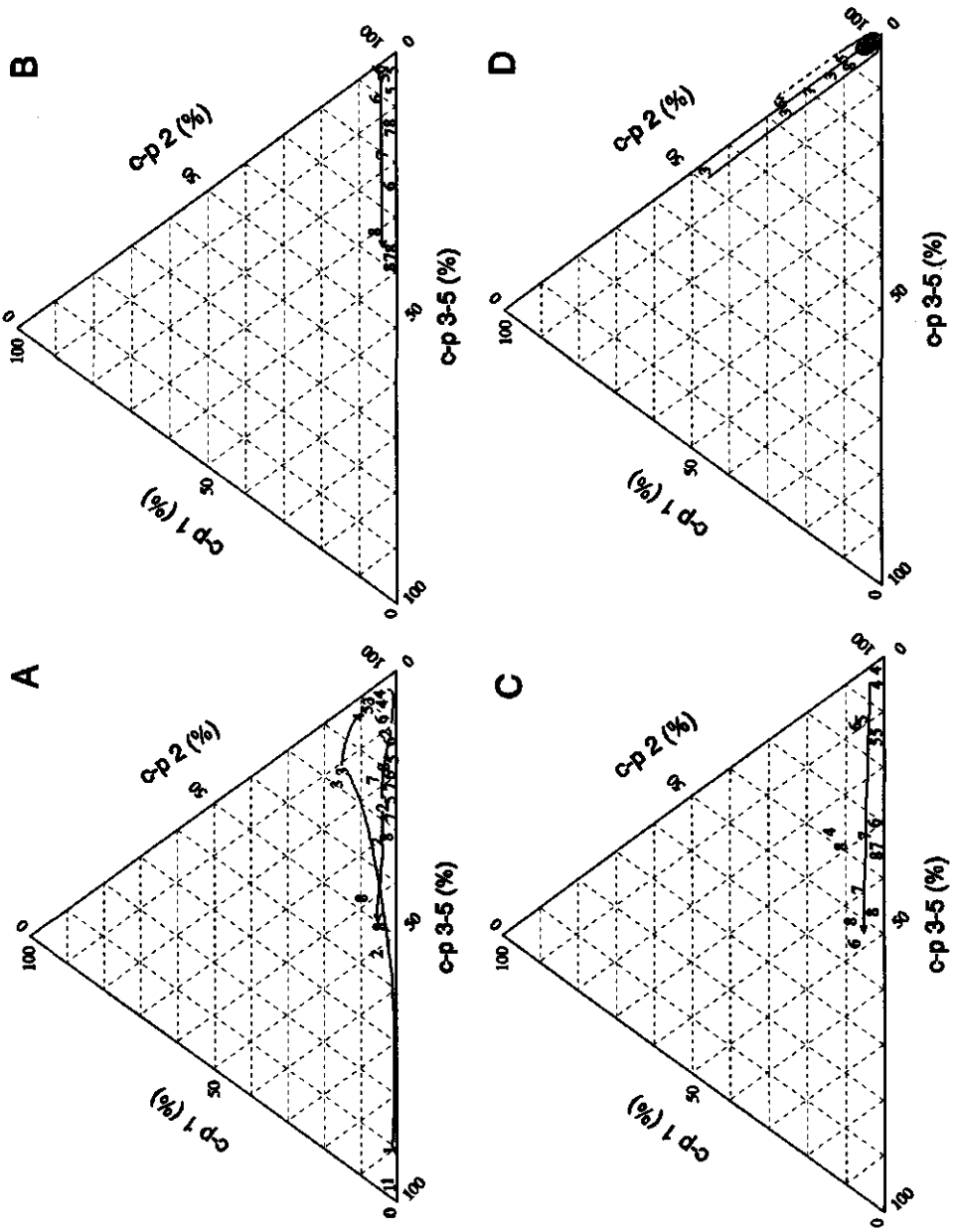
greater proportion of taxa which had to colonize the stages 3-4 belonged to c-p classes 1 or 2 (colonizers, opportunists) (55 and 79% respectively). this illustrates the colonizing potential within the type *i)* and/or *ii)* group of taxa (Table 5.4). Only 33% of the taxa which were restricted to the forested stages 5-8 belonged to c-p classes 1-2 (Table 5.4).

Colonization of the soil horizons by nematodes is exemplified by changes in c-p value distribution of nematode fauna (excluding root feeding nematodes) during horizon development (Fig. 5.6). In the first representatives of the various soil horizons, greater part of the non-root feeding nematode fauna consisted of opportunistic taxa, with c-p score 1 (litter horizon; Fig. 5.6.D) or c-p score 2 (fermentation and humus horizon; Fig. 5.6.B, C). However, during the subsequent development of the fermentation and humus horizons, a relative increase of persisters or K-strategists *sensu latu* (c-p group 3-5) was observed. In the 0-10 cm mineral soil similar trends were observed after first establishment of an organic horizon (Fig. 5.6.A). In the litter horizon, no population of persisters was found to develop, but taxa with c-p score 2 dominated the older stages.

In the course of succession, the total number of nematodes found in the profile (0-10 cm mineral soil including the horizons on top) increased from $56x10^3$ in stage 1 to $6078x10^3$ m^{-2} in stage 8 (Table 5.2). Also in the fermentation horizon, highest numbers of nematodes were found in the oldest stages. However, nematode abundance in the 0-10 cm mineral soil and litter horizon decreased in the stages 7-8 compared to the preceding stages. No trends were found in the humus horizons. In the stages 7 and 8 80-82% of the total nematode population in the profile occurred in the fermentation horizon, whereas 10-13% were found in the humus horizon, 4-5% in the 0-10 cm mineral soil and 1-5% in the litter horizon.

DISCUSSION

The primary succession of the vegetation, the nutrient dynamics within the vegetation and the organic profile of the 'Hulshorst sand' and 'Leuvenum forest' are well documented. The present study of the nematode fauna is the first on animal populations. Changes in the composition of the nematode fauna in the course of primary succession in a drift sand landscape appeared to be related to changes in vegetation and the development of an organic soil profile. During succession from bare drift sand to forest, different phases in nematode fauna composition could be recognized: i. bare drift sands without vegetation present, ii. vegetation of higher plants, but without trees , iii. Scots pine trees or forest.


B
c-p 2 (%)
c-p 1 (%)
c-p 3-5 (%)
D
c-p 2 (%)
c-p 1 (%)
c-p 3-5 (%)
A
c-p 2 (%)
c-p 1 (%)
c-p 3-5 (%)
C
c-p 2 (%)
c-p 1 (%)
c-p 3-5 (%)

The nematode fauna of the bare drift sands was dominated by the genus *Eudorylaimus*. Species of this genus are classified as omnivores (Yeates *et al.* 1993a) and because of their common occurrence in mosses and air-borne dust (Orr & Newton 1971, Krnjaic & Krnjaic 1972, Zullini & Peretti 1986) are expected to survive dehydration. During midsummer, maximum temperatures as well as temperature fluctuations in the top layer of the bare sand will be most extreme. Only well-adapted species can survive these climatic conditions. However, in autumn when maximum temperatures are lower, other taxa would also be able to survive in the top layer of the bare drift sand. It is not known whether they colonized the 0-10 cm drift sand from deeper soil horizons or relied on dispersal by wind each year. Except for the surface of the drift sand, where layers of algae can be found (Prach 1989), the low water table and organic matter content $\leq 0.4\%$ (Emmer *et al.* 1991) mean food conditions are very poor, especially in deeper soil layers. Thus, wind dispersion seemed most likely, as was shown by Orr and Newton (1971) for some of the taxa present in the October samples (*Aphelenchoides, Rhabditis, Ditylenchus*, Tylenchidae and Cephalobidae). The occurrence of the plant feeding ectoparasite *Trichodorus* in the October samples of the bare drift sand, supports this view.

The development of a vegetation composed of higher vascular plants, coincided with increased absolute nematode abundance and diversity. Introduction of trees in the primary succession led to further major shifts in nematode fauna composition. Some genera (*Acrobeles* and *Hemicycliophora*) could not be detected in the first stage where trees were present. They were not found in the third stage of sere B, where samples were taken only under the canopy of 3-5 year old Scots pine trees. However, they were present in the third stage of sere A, where the 0-10 cm mineral soil was also sampled outside the canopy. Other taxa (cf *Allantonema, Aporcelaimellus* and *Zeldia*) characteristic of the treeless vegetation also occurred under the canopy of the 3-5 year old Scots pine trees, but were absent in the subsequent successional stages (age of trees $\geq$25-30 years). In addition, taxa like *Wilsonema, Tylolaimophorus, Filenchus b, Steinernema* and *Teratocephalus* only occurred in the forested stages, where they were generally present.

In Chapter 4 relationships between the composition of the nematode fauna of a variety of habitats within the Netherlands and the pedological and floristic characteristics of those habitats were studied, and there also significant differences between forested and

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◂*Legend* Figure 5.6. C-P triangles of successional changes in nematode fauna composition within the 0-10 cm mineral soil (a), humus (b), fermentation (c) and litter (d) horizons of primary succession sere B. Numbers refer to the corresponding stages of succession. Trends are indicated (arrows). The samples which are not shown separately in Fig 5.6.d lay all within the hatched area.

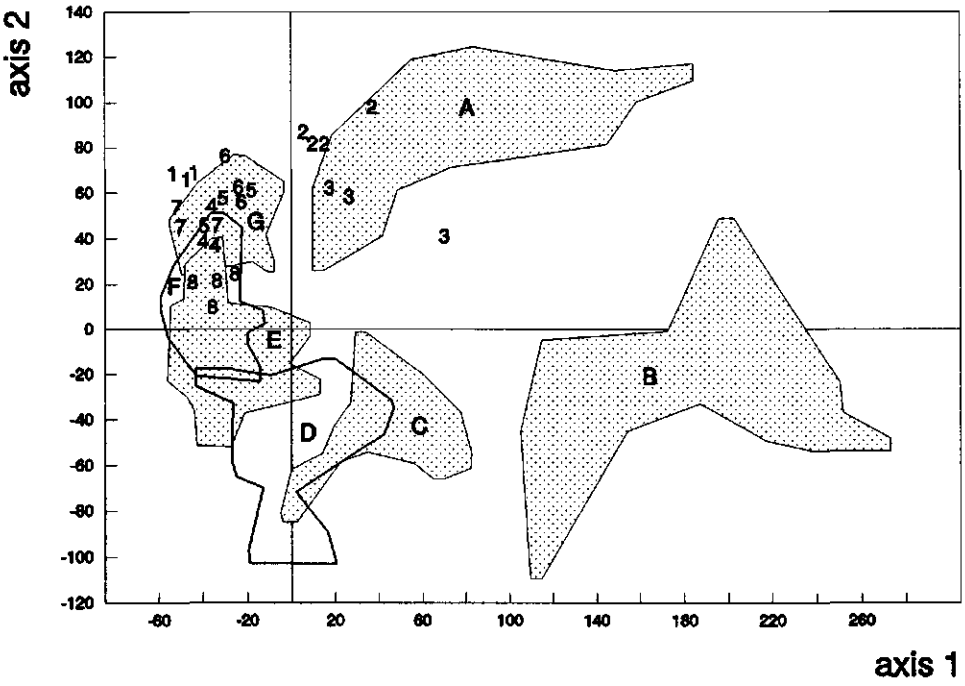

Figure 5.7. Ordination diagram (detrended correspondence analysis, DCA) of the axes 1 and 2 based on the nematode fauna of the 0-10 cm mineral soil of 209 nematode samples from a variety of habitats within the Netherlands (Chapter 4). Seven clusters of samples (A-G), each characterized by a specific combination of soil and floristic properties, are indicated. The 26 samples of the 0-10 cm mineral soil of sere B were included passively in the DCA. Their position within the diagram is indicated by numbers corresponding to the stage of succession.

unforested habitats with soils of comparable texture were found. When the nematode samples from 0-10 cm mineral soil of sere B were projected onto the correspondence analysis graph of the axis 1 and 2 of the data of Chapter 4, they coincided with those clusters composed of samples with related habitat types (Sample Groups; Fig. 5.7). Stages 2 and 3 coincided with Sample Group A, which is composed of grassland and dwarf-shrub vegetations on sandy soils. Stages 1 and 4-7 were positioned onto Sample Group G, the Scots pine forests. Stage 8, an early variant of a *Betulo-Quercetum*, was separated from the stages 1, 4-7 and coincided with Sample Group F. Just within this Sample Group F, the majority of sites with a *Betulo-Quercetum roboris* were classified (Chapter 4). These similarities between nematode community structure and habitat type as found in both investigations, support the classification of soils based on their nematode fauna as proposed in Chapter 4. Nematode sampling in Chapter 4 was restricted to the 0-10 cm

mineral soil. From our results it can be seen that as a consequence of such a restriction, the major part of the nematode fauna of soils with a mor type humus will be excluded from the classification, and this may result in lower discrimination between some successional stages.

Fanta (1986) found that, mainly due to game browsing, natural succession of the Scots pine forest towards a poor variant of birch-oak forest (*Betulo-Quercetum*) does not occur in the 105 year old stand of De Stille Eenzaamheid (stage 7). Our results showed successional relationships between the nematode fauna of stage 8 (a mixed Scots pine-birch-oak forest with a herb layer dominated by *V. myrtillus* and *E. nigrum* located in the same area), a possible example of subsequent development of the Scots pine forest towards a vegetation characterized by the presence of deciduous trees, and the preceding Scots pine stages. Moreover, Fig. 5.7 showed high similarity between the nematode fauna of this stage and other *Betulo-Querceta*. Reconstruction of the geological development, former vegetation and chemical-physical soil properties of site 8, are needed to determine its successional status.

As 67% of the nematode taxa of the 0-10 cm mineral soil of sere B were restricted to either the stages 2-3 or to the forested stages ($\geq$4), it is likely that some common environmental parameters determined the differences in nematode community structure between the unforested and forested habitats. Although specific causal connections are difficult to demonstrate, development of the vegetation and derived changes in microclimate and distribution of organic matter within the soil, will represent the driving forces. This is illustrated by the distribution of the plant feeding nematode taxa over both habitat types. Of the 16 plant feeding nematode genera which occurred in the 0-10 cm mineral soil of the stages 2-8 of sere B, 31 and 50% were restricted to the unforested and forested habitats respectively (Chapter 6). Only three plant feeding taxa occurred in both habitats, and of those only *Tylenchorhynchus microphasmis* was common throughout sere B. Furthermore, application of results of a preliminary study of the sensitivity of nematodes to dehydration (Booyink pers. comm.) to the data of sere B, showed a relative increase of taxa sensitive to dehydration in the deeper soil horizons. The observation of a similar increase of taxa sensitive to dehydration when going from the 0-10 cm mineral soil of the unforested stages to the forested stages, suggests influences of microclimatological nature, which will be associated with soil and vegetation development.

After Scots pine trees invaded the *Spergulo-Corynephoretum*, parallel to the progression of the vegetation succession, a mor type organic horizon started to develop. Besides implications of the presence of an organic horizon on the occurrence of

nematodes discussed above, the occurrence of nematodes will also depend on the morphological differentiation and subsequent changes in chemical-physical properties of the organic horizons (Emmer *et al.* 1991). Wasilewska (1970) who also studied the nematode fauna of Scots pine forests of different age, concluded that the colonization of nematodes in the forested habitat was primarily influenced by the cover with vascular plants in combination with the humus content of the soil. Above certain threshold settlement conditions, she observed a clear increase in number of nematode taxa. Such an increase in nematode genera was observed also in the 'Hulshorst sand' sere B by the change from stage 4 to stage 5, and was brought about mainly because of the development of the organic horizon. Stage 5 was the first successional stage in which all three master organic horizons (L, F and H horizon; Klinka *et al.* 1981) could be distinguished. In the subsequent Scots pine stages the total number of genera remained fairly constant, as was found also by Wasilewska (1970), but shifts in the composition of the nematode fauna still occurred (Figs 5.4, 5.5).

The assumption of a dominant influence by the stage of development of the organic layer on the occurrence of nematodes, is further supported by changes in c-p value group distribution within the various soil horizons. Relating the colonizing abilities of the nematode taxa, as expressed by their c-p score (Bongers 1990b), with the stage of development of the various soil horizons, showed that the nematode faunae of the initial stages of the master horizons distinguished were composed mainly of taxa characterized as opportunists (c-p score 1 or 2). These taxa are among the first to respond to increased food availability or to colonize vacant niches (Bongers 1990b, Bongers *et al.* 1991). In the next stages the relative importance of persisters or K-strategists *sensu latu* increased, a trend which was observed also in the 0-10 cm mineral soil after first establishment of an organic horizon. However, such a development of persisters was not found for the litter horizon. Moreover, here relatively large numbers of the opportunist *Panagrolaimus* (c-p score 1) were found also in the older stages, indicating unstable and/or temporarily *ad libitum* food sources. Thus, the initial nematode fauna of the various horizons was characterized by a considerable degree of opportunism (Swift *et al.* 1979). However, in the litter horizon this was found to be independent of the stage of succession, which probably is related to varying food and feeding conditions in this horizon.

The soil profile was characterized by distinct morphological differences between litter, fermentation and humus horizons, and a sharp change from the organic horizon to mineral soil. Moreover, when going from litter via fermentation to humus, pH decreased and bulk density increased, indicating that the morphological differentiation paralleled chemical and physical differentiation (Emmer *et al.* 1991). The correspondence analyses and similarity measurements showed that comparable soil horizons of the older ($\geq$ stage

5) forested sites, had higher similarity than different horizons within the same profile (Figs 5.3, 5.4). Arpin and Ponge (1986) achieved similar results when studying the nematode fauna of *P. sylvestris* and *Quercus petraea* plantations and a mixed plantation of these tree species. Thus within each master organic horizon as well as in the 0-10 cm mineral soil a nematode fauna developed, which can be related to specific environmental conditions of the horizon. Nematode taxa composition (e.g. in the litter horizon) appeared to be mainly due to food conditions (Chapter 6) and resistance to adverse microclimatological characteristics (e.g. dehydration). As far as species were identified, none of the common species of the deeper soil horizons were common in the litter layer (Chapter 6). However, such an absolute segregation in species distribution was not found for the deeper horizons, although some species were restricted to a selection of horizons (Chapter 6). Thus, in addition to "lateral orientated habitats" for soil animals (Emmer *et al.* 1991), vertical migration of certain nematode species, as probably can be derived also from the colonization process of the fermentation and humus horizons in the course of succession, cannot be ignored.

ACKNOWLEDGEMENTS

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NEMATODE DISTRIBUTION, TROPHIC STRUCTURE AND BIOMASS IN A PRIMARY SUCCESSION OF BLOWN-OUT AREAS IN A DRIFT SAND LANDSCAPE

SUMMARY

The nematode fauna of a successional sere in a blown-out area in a drift sand landscape, including drift sands without vegetation, Spergulo-Corynephoretum *and Scots pine (*Pinus sylvestris *L.) forests of different ages, was studied. Samples were taken from the 0-10 cm mineral soil and, in the forested stages, also from the organic layers. The organic layers were divided into litter, fermentation and humus horizons. The initial stages of succession were dominated by omnivorous nematodes. Nematodes feeding on lower plants were restricted to the surface layers and different species occurred during the succession. In litter horizons nematode trophic structure followed microfloral succession. The occurrence of plant feeding nematodes was related to vegetation. Vertical distribution patterns of plant feeding nematodes could be related to rooting patterns of herb and tree species. Bacterial and plant feeding nematodes reached highest densities and biomass* m^{-2} *in the fermentation horizons and increased during succession. Predatory nematodes were rare and occurred only in mineral soil. Average nematode length differed by soil horizon and decreased in the 0-10 cm mineral soil during succession. Relationships between nematode morphometrics and micro-climatological conditions are discussed.*

INTRODUCTION

Short-term nematode population dynamics has been studied extensively during the past two decades. For the temperate regions, annual nematode population dynamics have been described for various natural ecosystems, including grasslands, deciduous and coniferous forests, bogs and heathland (Peterson & Luxton 1982). Grouping nematode species into ecological groups, mostly based on their feeding requirements, and subsequent relating of the abundance and biomass of these groups to other soil biota and ecological soil processes, has given insight to the position of nematodes in soil processes. In contrast, data on long-term changes in the occurrence of nematodes in relation to habitat development are scarce, but are valuable both to the understanding of changes in soil ecological processes during habitat development and to evaluation of the ecological condition of soils ("soil health") (Chapter 5).

Only in Wasilewska (1970, 1971) and Chapter 5 descriptions of nematode community development in relation to successional changes in temperate forests have been given.

Vegetation succession of afforested dunes paralleled increasing nematode diversity, abundance and biomass, and decreasing average nematode body weight (Wasilewska 1970, 1971). Furthermore, shifts were observed in feeding group proportions; a relative increase of 'facultative' plant feeding nematodes as compared with obligatory plant feeders, and a decreasing importance of omnivorous nematodes being the most marked changes. Wasilewska (1971) concluded that these changes were related to differences in plant cover and humus content of the successional stages. In Chapter 5, where nematode community structure in a primary succession of blown-out areas in a drift sand landscape was studied, a comparable increase in nematode diversity during forest succession was found. This increase was related to the development of the organic horizon. As succession proceeds on these soils, the organic horizon of Scots pine forests (the primary colonizing tree species) develops into a mor type organic profile (Emmer *et al.* 1991). At full development within such an organic profile litter, fermentation and humus horizons, which reflect different stages of decomposition of the organic matter, can be recognized (Klinka *et al.* 1981). The maximum number of nematode species coincided with the first occurrence of a fully developed mor humus (Chapter 5). Each horizon was also characterized by its nematode faunal composition. These changes in nematode species distribution during succession and within the soil profile, showed relationships to vegetation and micro-climatological conditions, and indicated significant differences in ecological conditions between the various soil horizons.

This chapter describes the trophic group distributions, the spatial and successional species distributions, and the total biomass and body dimensions of the nematode fauna of the primary succession of blown-out areas in a drift sand landscape described in Chapter 5. Effects of succession on the nematode community are discussed with emphasis on the ecological processes with which the various trophic groups are associated, and changes in habitat conditions related to the development of the organic horizon and the vegetation.

MATERIALS & METHODS

The results presented are based on the nematode samples taken from successional sere B near Harderwijk in the Netherlands, as described in Chapter 5. Briefly summarizing, the nematode fauna of eight successional stages, characterized as (1) bare drift sand, (2) *Spergulo-Corynephoretum*, Scots pine (*Pinus sylvestris* L.) forests of respectively (3) 3-5, (4) 25-30, (5) 45-50, (6) 80-90 and (7) 105 years old and (8) an early variant of *Betulo-Quercetum* with age comparable to stage 7, were investigated. At each site the 0-10 cm mineral soil was sampled and, depending on the state of development of the organic horizon, samples were taken from the litter, fermentation and humus horizons. No significant differences in thickness of each of the organic horizons were found between the successional

stages. Thickness of the fermentation and humus horizons was (mean and standard deviation in parentheses) 6.1 (1.28) and 1.7 (0.69) cm respectively; thickness of the litter horizon was <0.5 cm.

At stage 3 no herb layer but only pine needles were present. The herb layer of stage 4 was composed of *Festuca ovina* L., *Corynephorus canescens* (L.) Beauv. and *P. sylvestris* needles. From stage 5 onwards the herb layer was composed of *Deschampsia flexuosa* (L.) Trin. and mosses, with in the stage 7 also *Empetrum nigrum* L., *Vaccinium myrtillus* L. and seedlings of *Prunus* sp., *Betula* sp. *Quercus* sp. and *Sorbus aucuparia* L.. *E. nigrum* and *V. myrtillus* dominated the herb layer of stage 8, whereas *D. flexuosa* and mosses were present in low densities.

Adult nematodes were identified to species level. However, as in some samples juveniles could not adequately be attributed to species, because of either the lack of adults or the presence of mixed species populations, the results will generally be presented at genus level. Allocation of nematodes to trophic groups followed Yeates *et al.* (1993a). Nematodes feeding by piercing algae, lichens or mosses and those feeding on unicellular eucaryotes were combined into one group: nematodes feeding on lower plants. The taxa found (see Bongers (1988) for authorities), with their trophic habit are (for plant feeding nematodes subgroups are indicated by a. sedentary parasites, b. migratory parasites, c. semi-endoparasites, d. ectoparasites and e. epidermal and root hair feeders):
Plant feeding nematodes: *Aglenchus agricola* (e), *Cephalenchus hexalineatus* (d), *Criconema sphagni* (d), *Filenchus ditissimus* (e), *F. helenae* (e), *Hemicycliophora epicharoides* (d), *Heterodera* sp. (a), *Malenchus bryophilus* (e), *M. sulcus* (e), *Merlinius microdorus* (d), *Merlinius* sp. (d), *Nagelus obscurus* (d), *Paratrichodorus teres* (d), *Pratylenchus* sp. (b), *Rotylenchus* sp. (c), *Tylenchorhynchus microphasmis* (d) and *Tylenchorhynchus* sp. (d); Hyphal feeding nematodes: *Aphelenchoides* spp., *Deladenus durus, Ditylenchus* spp., *Paraphelenchus pseudoparietinus, Pseudhalenchus minutus, Tylencholaimus mirabilis* and *Tylolaimophorus typicus;* Bacterial feeding nematodes: *Acrobeles mariannae, Acrobeloides nanus, A. tricornis, Alaimus mucronatus, Bunonema richtersi, Cephalobus* sp., *Cervidellus serratus, Cylindrolaimus* sp., *Domorganus* sp., *Drilocephalobus* sp., *Eucephalobus mucronatus, Eumonhystera vulgaris, Heterocephalobus elongatus, Metadiplogaster* sp., *Metateratocephalus crassidens*, Nematode A (undescribed alaimid), *Panagrolaimus rigidus, Plectus acuminatus, P. longicaudatus, P. pusillus, Prismatolaimus intermedius, Prodesmodora* sp., *Protorhabditis* sp., *Rhabditonema propinquum, Steinernema* sp., *Teratocephalus* sp. *Wilsonema otophorum* and *Zeldia punctata*; Predators: *Seinura* sp. and *Tripyla* sp.; nematodes feeding on lower plants: *Laimaphelenchus penardi, L. pini, L. pannocaudus* and *Tylenchus davainei*; Omnivores: *Aporcelaimellus obtusicaudatus, Aporcelaimium* sp. and *Eudorylaimus* sp..

From every successional stage, the total length (L, μm) and longitudinal section (A, μm^2) of 50-60 randomly selected nematodes were measured using a GOP 302 image-analyzer (Context Vision, Sweden). Nematode biomass (G) was calculated from the Andrássy equation $G(\mu g)=(W^2xL)x(16x10^{-5})$. Body width W($\mu$m) was calculated as the A/L ratio, and therefore consequently was an underestimation of maximum body width, which is generally used in the Andrássy equation. In principle the Andrássy equation is based on nematode volume multiplied by a constant ($1.0625x10^{-6}$). Robinson (1984) showed that nematodes' volume calculated using the area/length ratio, resulted in an underestimation of nematode volume in the range of 3-12%, whereas bias in the Andrássy method ranged from -2 to +23%. Thus biomass estimates presented in this study will be on average 0-28% lower than those obtained using W.

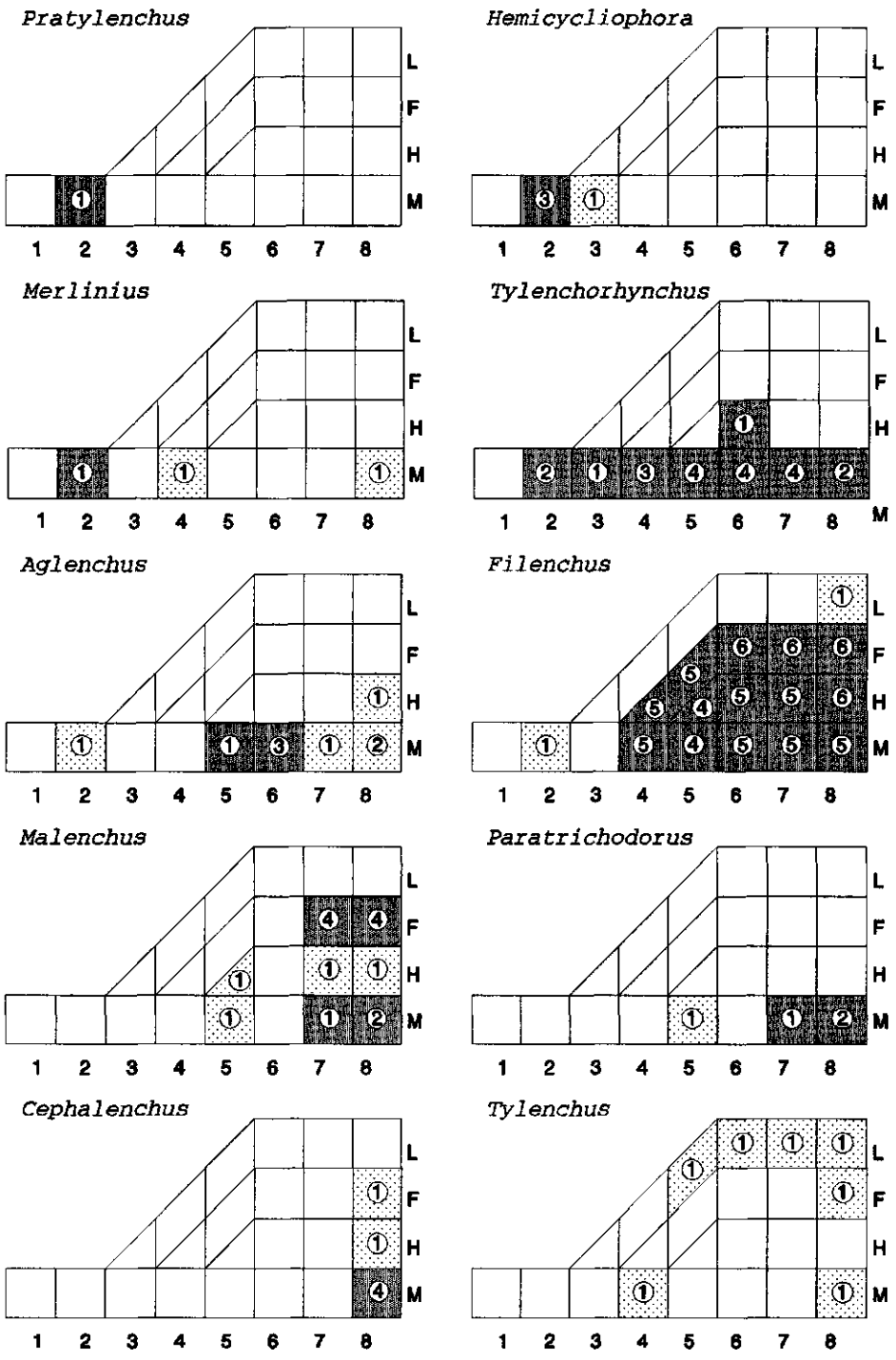
Differences in trophic group composition between the various successional stages were tested by the Mann-Whitney U-test. Body length was log transformed, and differences in mean population body length between the various successional stages and soil horizons were tested by an analysis of variance.

RESULTS

Genera and trophic group distribution

Omnivorous nematodes, represented by three genera only, dominated the early successional stages, especially the drift sands where no vegetation was present (Fig. 6.1). *Aporcelaimellus* and *Aporcelaimium* (present only in stage 3) predominantly occurred in the 0-10 cm mineral soil of the *Spergulo-Corynephoretum* and under the invading young Scots pine trees. On the other hand, *Eudorylaimus* was present throughout most of the succession, and occurred both in the mineral soil and the organic horizons. The absence of omnivorous nematodes in the samples taken in successional stage 4 is remarkable (Table 6.1, Fig. 6.1). The discontinuous distribution of the genus *Eudorylaimus* with respect to succession indicates that several species may be involved. In the forested stages, *Eudorylaimus* reached highest

Legend Figure 6.1. Vertical distribution of 40 common nematode genera in stages 1-8 of a primary succession on blown-out areas on a drift sand. Soil horizons are litter (L), fermentation (F), humus (H) and 0-10 cm mineral soil (M). Indicated is whether genera were absent (white), present in $\leq$50% of the samples (stippled), or present in >50% of the samples (solid). Average nematode population density is given by relative abundance classes: 1 <0;2], 2 <2;4], 3 <4;8], 4 <8;16], 5 <16;32], 6 <32;64] and 7 <64;100] (n=3 for the stages 1, 3-7 and n=4 for the stages 2 and 8).



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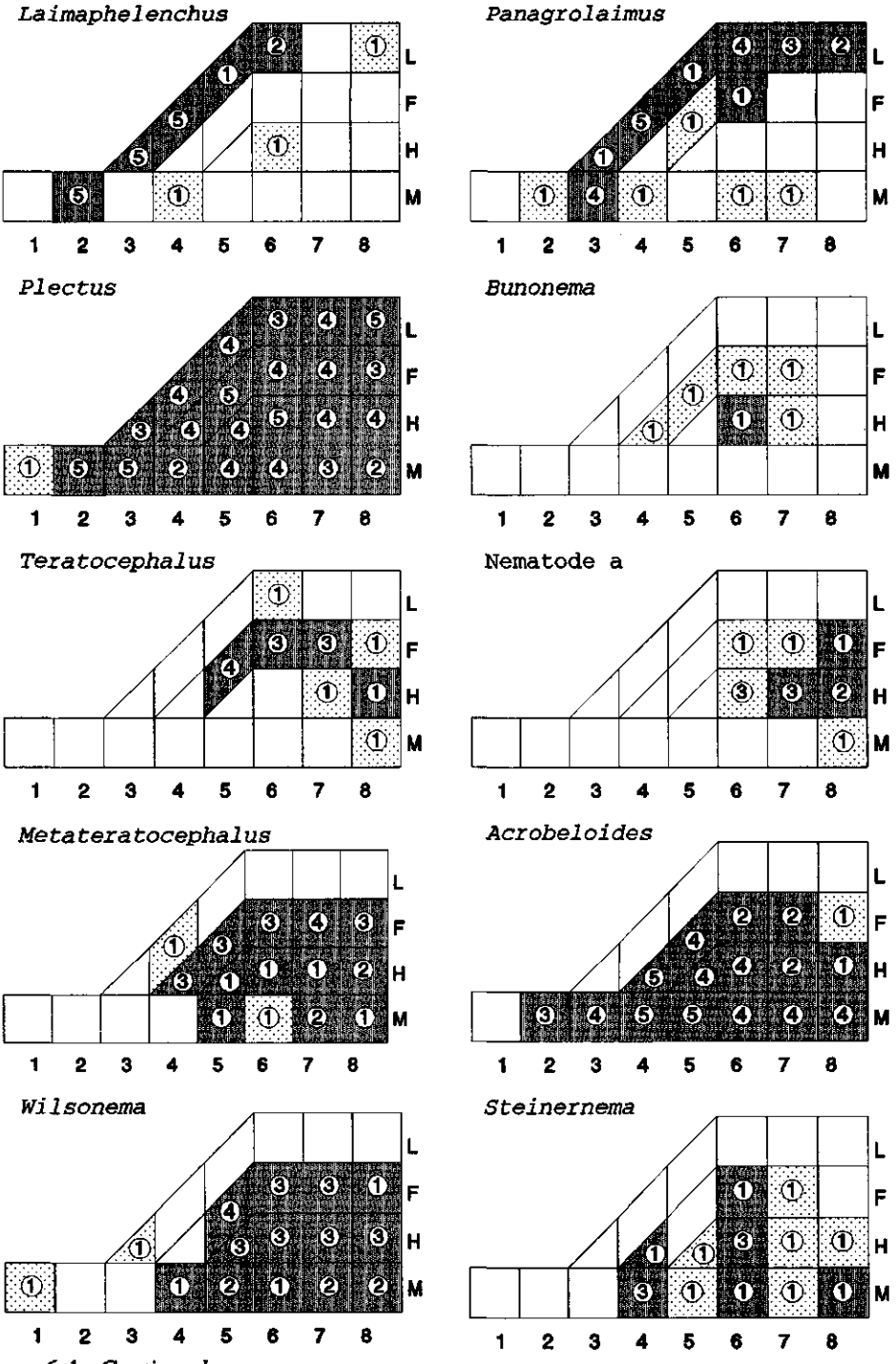

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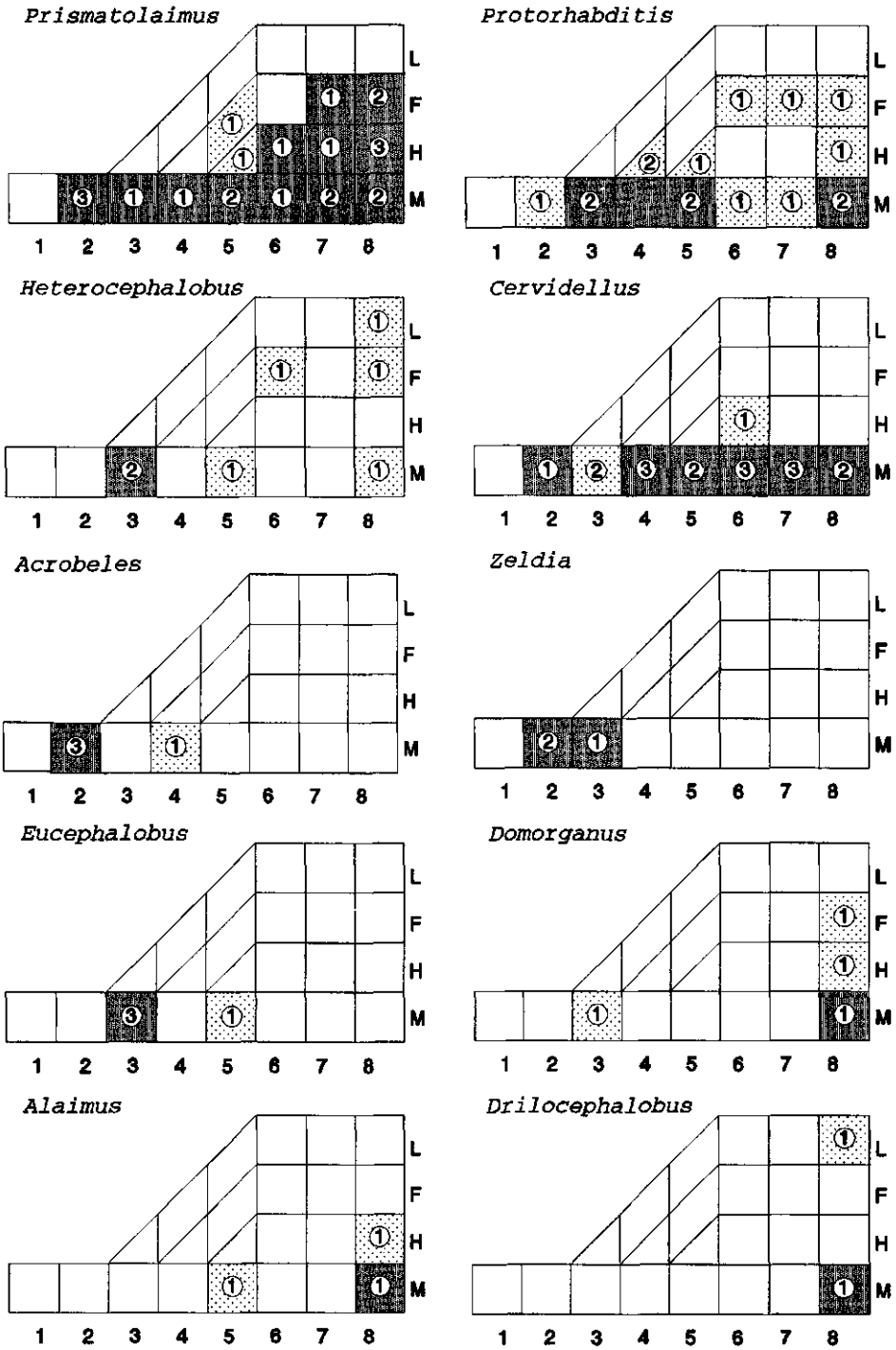

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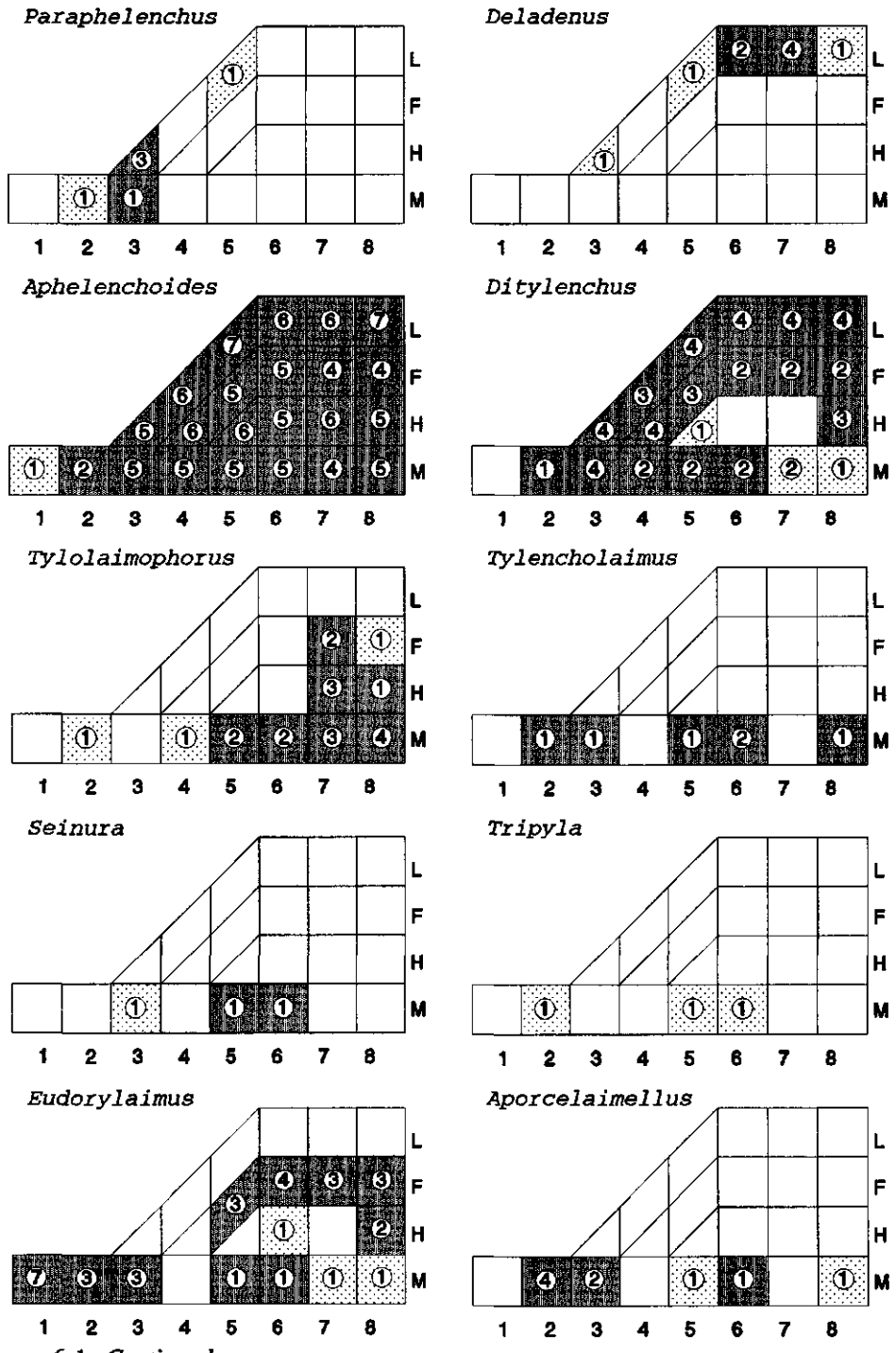

Figure 6.1. *Continued*

densities in the fermentation horizon, with their numbers increasing in this horizon during the succession.

Laimaphelenchus and *Tylenchus* (nematodes feeding on lower plants) mainly occurred in the superficial soil horizons (Fig. 6.1, Table 6.1), corresponding to the distribution of mosses, lichens and algae. They reached highest densities in the *Spergulo-Corynephoretum*. The sharp decrease in numbers in stage 5, coincided with the closure of the herb layer (in stage 3 and 4 still >30-50% of the area was pine litter). The species of *Laimaphelenchus* showed different optima in the succession, with *L. pini*, *L. penardi* and *L. pannocaudus* having their peak occurrence in stage 2, 3-4 and 5 respectively (Fig. 6.2). *Tylenchus* replaced *Laimaphelenchus* in the oldest successional stages.

Together with the nematodes feeding on lower plants, the bacterial feeding nematodes dominated the nematode community of the *Spergulo-Corynephoretum* where the two groups composed 23 and 45% of the total numbers respectively (Table 6.1). Only three of the total 25 bacterial feeding genera found in this study were restricted to the first successional stages (*Acrobeles, Zeldia, Eucephalobus*) (Fig. 6.1).

The abundance and relative proportion of the bacterial feeding nematodes depended both on the soil horizon and on the stage of succession. In the 0-10 cm mineral soil, their numbers remained fairly constant ($178\text{-}299 \times 10^3$ m^{-2}) until stage 6 (the 80-90 years old Scots pine forest), but decreased significantly to $61\text{-}76 \times 10^3$ m^{-2} in the older forests. The highest abundance of bacterial feeding nematodes was found in the fermentation horizon, where numbers increased from 221×10^3 in stage 4 to 858×10^3 m^{-2} in the *Betulo-Quercetum*. However, in this horizon the proportion of bacterial feeding nematodes reached a peak in stage 5 (50%) and decreased subsequently to 27-31% in the oldest stages. This was mainly due to a strong increase in plant feeding nematodes as succession proceeded.

In the litter horizon the hyphal feeding nematodes outnumbered the bacterial feeding nematodes. Here the bacterial feeders:hyphal feeders (BF:HF) ratio was 0.2-0.3 (exceptionally 0.6 at stage 3), whereas it was ≥ 1 in the 0-10 cm mineral soil and in most of the samples taken from the fermentation horizon (see below for exceptions). Only two bacterial feeding genera (*Panagrolaimus*, *Plectus*) were common in the litter horizon. The distribution of *Panagrolaimus* was mainly restricted to this layer, whereas *Plectus* occurred throughout the profile. However, some spatial segregation of the three *Plectus* species present in this study, seemed to occur (Fig. 6.2); *P. acuminatus* and possibly also *P. pusillus* predominantly occurred in the surface layers, whereas *P. longicaudatus* was rare in the litter horizon, but common in the deeper horizons. Most other bacterial feeding genera also showed characteristic distribution patterns within the soil profile and/or succession. In the forested stages *Bunonema*, Nematode A and *Teratocephalus* were found mainly in the

fermentation and humus horizons. *Cervidellus* and *Eucephalobus* were mainly restricted to the 0-10 cm mineral soil, whereas *Wilsonema, Metateratocephalus, Acrobeloides, Prismatolaimus, Protorhabditis, Heterocephalobus* and *Steinernema* were found both in the 0-10 cm mineral soil and in the fermentation and humus horizons. *Alaimus, Domorganus, Drilocephalobus* and *Prodesmodora* were found mainly in the oldest successional stages.

The distribution of six of the eight hyphal feeding genera is shown in Fig. 6.1. *Aphelenchoides* occurred in all horizons and in all stages. The only other genus with such an extensive distribution was the bacterial feeding *Plectus*, and as with *Plectus* several species of *Aphelenchoides* were involved (Fig. 6.2). The hyphal feeding *Ditylenchus* had a similar distribution to *Aphelenchoides*, but was found less frequently in the humus horizon. *Paraphelenchus* and *Deladenus* occurred in the topmost horizons, with peak occurrences in stage 3 and 6-7 respectively. The remaining hyphal feeding genera predominantly occurred in the 0-10 cm mineral soil. Among these, *Tylolaimophorus* reached highest relative abundances in the oldest stages [stages (6)7-8], where it also occurred in the fermentation and humus horizons.

The absolute numbers of hyphal feeding nematodes were highest in the oldest successional stages (stages 5-8), mainly because of increasing numbers in the organic horizon (Table 6.1). In the 0-10 cm mineral soil maximum abundances were found in the stages 3-6. In the litter horizon the hyphal feeding nematodes were the most abundant trophic group, where they composed 45-84% (weighted mean 70%) of the total nematode fauna. In all other horizons their proportion was $\leq$45% (one exception: humus horizon of stage 5, proportion 60%). The relatively high proportion of hyphal feeding nematodes in the first stages of the various organic horizons as well as in the 0-10 cm mineral soil of stage 3 as compared to the same horizons of the subsequent stages is notable (fermentation horizon stage 4 and 5-8, 41 and 17-24% respectively; humus horizon stage 5 and 6-8, 60 and 24-45%; 0-10 cm mineral soil stage 3 and 4-8, 40 and 22-28%). However, no significant differences in absolute numbers were found (Table 6.1); thus the relatively high percentages of hyphal feeding nematodes resulted from changes in other trophic groups.

An exceptionally high BF:HF ratio (mean and standard deviation in parentheses) of 9.7 (4.52) was found for the 0-10 cm mineral soil of the *Spergulo-Corynephoretum*, as the second highest value was only 2.4 (1.40) (fermentation horizon stage 5).

Invasion by Scots pine and related changes in the composition of the vegetation, paralleled profound changes in the occurrence of the plant feeding nematodes. The numbers of plant feeding nematodes decreased significantly from $48x10^3$ m^{-2} in the *Spergulo-Corynephoretum* to only $6x10^3$ m^{-2} under the 3-5 year old Scots pine trees of stage 3 (Table 6.1). However, in the subsequent stages the numbers in 0-10 cm mineral soil increased to

Table 6.1. Mean densities (numbers x1000 m^{-2}) of trophic groups per soil horizon and stage of a succession.

TROPHIC GROUP	SUCCESSIONAL STAGE							
	1	2	3	4	5	6	7	8
Litter								
BF			65 *a*	29 *bc*	86 *a*	50 *ac*	21 *bc*	16 *bc*
HF			108 *ac*	132 *a*	336 *b*	205 *a*	126 *ac*	50 *c*
PF			0	0	0	0	0	<1
OV			0	0	0	0	0	0
PR			0	0	0	0	0	0
LF			67 *a*	35 *ab*	5 *bc*	6 *b*	2 *bc*	1 *c*
Fermentation								
BF				202 *a*	572 *b*	697 *bc*	730 *c*	1326 *bc*
HF				221 *a*	268 *ab*	414 *ab*	519 *ab*	858 *b*
PF				116 *a*	253 *ab*	752 *bc*	1010 *c*	2656 *d*
OV				0 *a*	43 *b*	77 *bc*	80 *bc*	130 *c*
PR				0	0	0	0	0
LF				0	0	0	0	30
Humus								
BF					83	226	82	147
HF					159	124	127	184
PF					23 *a*	121 *b*	74 *b*	421 *ab*
OV					0 *b*	4 *ab*	0 *b*	11 *a*
PR					0	0	0	0
LF					0	1	0	0
0-10 cm Mineral soil								
BF	1 *a*	209 *bd*	269 *b*	161 *bd*	178 *d*	299 *bd*	61 *c*	76 *c*
HF	1 *a*	27 *b*	183 *cd*	97*bcd*	110 *cd*	212 *c*	35 *b*	70 *d*
PF	0 *a*	48 *bd*	6 *a*	105*bcd*	116 *c*	285 *e*	59 *df*	97 *cf*
a-d	0 *a*	46 *bd*	6 *ac*	10 *c*	45 *bd*	72 *bd*	16 *b*	34 *d*
e	0 *a*	2 *a*	0 *a*	95 *bc*	71 *b*	212 *c*	43 *b*	62 *b*
OV	55 *ac*	62 *a*	25 *bc*	0 *d*	7 *bd*	13 *b*	1 *d*	3 *d*
PR	0 *ac*	9*abc*	3*abc*	0 *ac*	3 *b*	16 *ab*	0 *ac*	0 *c*
LF	0 *a*	106 *b*	0 *a*	2 *ab*	0 *a*	0 *a*	0 *a*	<1 *a*

Bacterial feeding (BF), hyphal feeding (HF), plant feeding (PF), omnivores (OV), predators (PR) and feeding on lower plants (LF); in the 0-10 cm mineral soil plant feeding nematodes are divided into endo- and ectoparasites (a-d) and epidermal and root hair feeders (e); in a given line numbers followed by different letters differ significantly ($p \leq 0.05$; one sided).

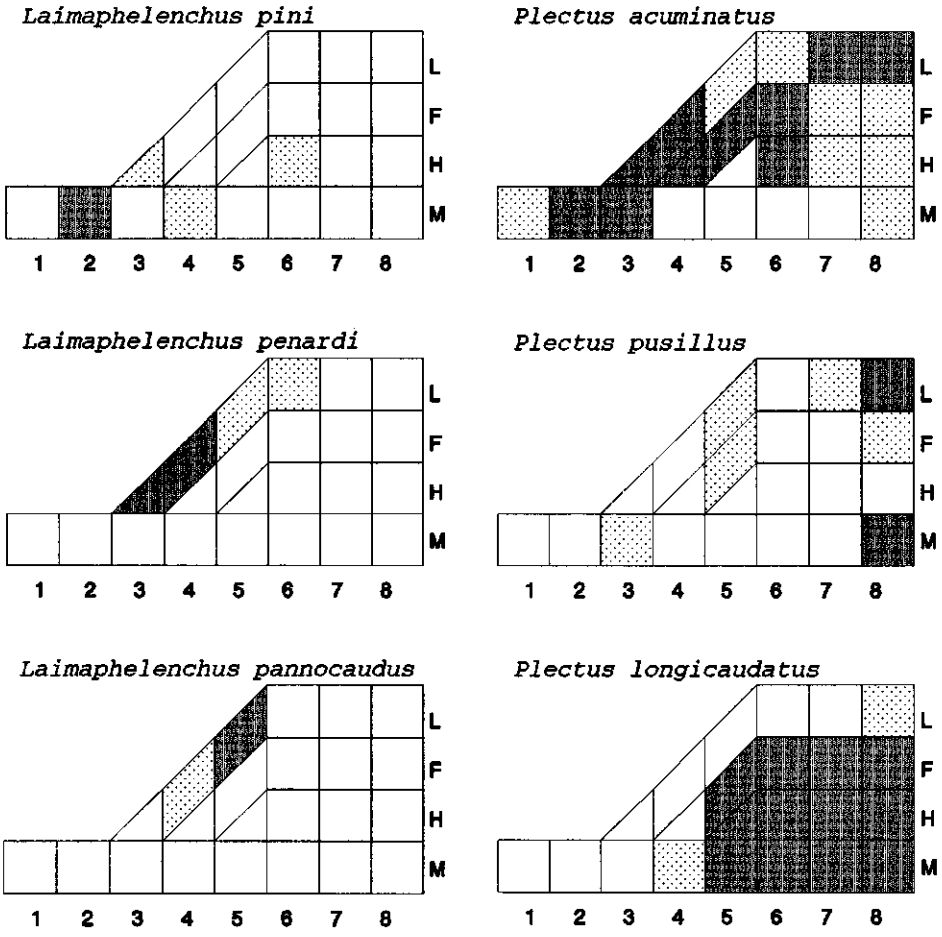

Figure 6.2. Vertical distribution of the species of *Laimaphelenchus* and *Plectus* in stages (1-8) of a primary succession on blown-out areas on a drift sand. See Fig. 6.1 for details.

a peak of 285×10^3 m^{-2} in stage 6, and then subsequently decreased again to $59\text{-}97 \times 10^3$ m^{-2}. In both cases the decrease in numbers was accompanied by shifts in the generic composition. With the development of the young forest, *Pratylenchus, Hemicycliophora, Merlinius, Nagelus* and *Tylenchorhynchus* decreased below detection level or disappeared, and populations of *Filenchus* and *Aglenchus* started to develop (Fig. 6.1). Greatest development of populations of *Paratrichodorus, Cephalenchus* and *Malenchus* were found after stage 6, whereas *Aglenchus* seemed to decrease. *Tylenchorhynchus microphasmis* is the only plant feeding genus which is common in all stages with vegetation present, both outside and inside

the forest.

The diversity of plant feeding nematodes in the organic horizons was relatively low. Of the fifteen plant feeding genera only *Filenchus* and *Malenchus* were frequently found in these horizons, where they were mainly responsible for a significant increase in absolute numbers of plant feeding nematodes, from $116x10^3$ m^{-2} in stage 4 to $2685x10^3$ m^{-2} in stage 8 (respectively 22 and 54% of total numbers in the fermentation horizon). Subdivision of plant feeding nematodes in groups based on aspects of their feeding biology, showed the almost exclusive occurrence of epidermal and root hair feeders in the fermentation and humus horizons (group e; Fig. 6.1). Plant feeding nematodes of groups a-d (sedentary parasites, migratory endoparasites, semi-endoparasites and ectoparasites respectively) were mainly restricted to the mineral soil where they occurred together with epidermal and root hair feeders. The latter were absent or rare in the 0-10 cm mineral soil of the early stages of succession (stages 2 and 3; Table 6.1).

Predatory nematodes were found only in 0-10 cm mineral soil and then only in stages 2-3 and 5-6 (Table 6.1, Fig. 6.1). Their population reached 16,000 m^{-2}, and they comprised 1-2% of the total nematode fauna of this horizon.

Table 6.2. Nematode biomass ($mg.m^{-2}$) in the different soil horizons of the various stages of a primary succession on blown-out areas on drift sand.

SUCCESSIONAL STAGE	SOIL HORIZON								
	L		F		H		M		T
1							47	(100)	47
2							99	(100)	99
3	32	(27)					85	(73)	117
4	17	(22)	31	(41)			30	(38)	79
5	32	(22)	67	(46)	8	(6)	39	(27)	146
6	23	(12)	75	(41)	38	(21)	49	(27)	185
7	14	(8)	138	(80)	13	(8)	9	(5)	173
8	5	(2)	243	(87)	13	(5)	19	(7)	280

Percentages within soil profile are given between brackets; Litter (L), fermentation (F), humus (H) horizon, 0-10 cm mineral soil (M) and total (T).

Table 6.3. Average length (μm) of nematodes per soil horizon in the various stages of a primary succession on blown-out areas on drift sand.

SUCCESSIONAL STAGE	SOIL HORIZON			
	L	F	H	M
1				890 *a*
2				484 *bc*
3	539 *b*			439 *ce*
4	455 *cd*	370 *fgh*		403 *def*
5	436 *ce*	362 *fgi*	264 *m*	389 *eg*
6	445 *cd*	325 *ijl*	342 *gl*	357 *fgj*
7	462 *c*	340 *hijkl*	274 *m*	341 *hijkl*
8	438 *ce*	326 *hijkl*	266 *m*	353 *gk*

Numbers within the table followed by different letters differ significantly ($p \leq 0.05$); see Table 6.2 for abbreviations.

Nematode biomass and length

In 0-10 cm mineral soil nematode biomass was highest in stages 2 and 3 (99 and 85 $mg.m^{-2}$ respectively) (Table 6.2). Relatively low values (< 20 $mg.m^{-2}$) were obtained for the oldest forests of stages 7 and 8. In the fermentation horizon a gradual increase in biomass from 31 $mg.m^{-2}$ in stage 4 to 243 $mg.m^{-2}$ in stage 8 was found, reflecting the increase in absolute numbers in this horizon. Compared with the absolute abundance of nematodes in the litter horizon, the total nematode biomass in this horizon was relatively high. The nematodes in the litter horizon were on average significantly longer than those in the underlying horizons (Table 6.3). With increasing depth nematode length decreased significantly in the organic horizons, with minimum lengths in the humus horizon. In the underlying 0-10 cm mineral soil, average length of nematodes was significantly larger than in the humus horizon, but it did not differ from the lengths in the litter layers of the stages 4 and 5 and the fermentation layers. Average length of nematodes in the mineral soil was maximum in the drift sands of stage 1, and decreased gradually with increasing age of the forests.

Longer nematodes tended to have greater body width; the average nematode width in stages 3-8, calculated as the A:L ratio, was 15.2, 12.6, 11.7 and 14.4 μm for the litter, fermentation and humus horizon and 0-10 cm mineral soil respectively. The relatively large average nematode length in the humus horizon of stage 6 compared with stages 5, 7 and 8

is remarkable. As a result of this and greater absolute nematode abundance, the total biomass of the nematode fauna of stage 6 was also relatively high (Table 6.2).

DISCUSSION

Successional changes in the vegetation and related soil development of the blown-out areas were correlated with changes in nematode community structure (Chapter 5). These successional changes in nematode community structure showed a pattern of initial dominance of nematode taxa generally characterized as colonizers (*sensu latu* Bongers 1990b), followed by the development of nematode faunae characteristic for the stage of succession and soil horizon (Chapter 5). The present study showed that, depending on the stage of succession, the nematode fauna of these soil horizons also had characteristic trophic structures (Table 6.1).

The surface layers of successional stages 2-8 were characterized by the occurrence of relatively high numbers of hyphal feeding nematodes and the presence of nematodes feeding on lower plants. Greatest abundances of the latter were found in stage 2 where algae, mosses and lichens constituted 39-98% of total biomass of the herb layer (Moszynska 1991). As total biomass of lower plants in the forested stages remained high (450-2170 and 560-8840 kg/ha for the forests and *Spergulo-Corynephoretum* respectively (Moszynska 1991)), this does not explain the decrease in numbers of nematodes feeding on lower plants and the disappearance of *Laimaphelenchus* as succession proceeded. However, the increasing cover of the herb layer and tree canopy during succession, are likely to depress the occurrence of photosynthetic unicellular organisms (e.g. algae) in the litter horizons, and the three *Laimaphelenchus* species found in this study were probably associated with such algae, as they are common inhabitants of algae layers covering tree trunks (Bongers 1988). On the other hand, *Tylenchus* specifically occurred in the late successional stages (stage ≥ 5) where it probably fed on mosses (Procter 1984, Yeates *et al.* 1993a).

The nematode fauna of the litter horizons was characterized by the extreme dominance of hyphal feeding nematodes (Table 6.1). Studies on the succession of microflora and soil organisms during the decomposition of pine needles, showed a dominance of fungi in the initial stages of decomposition (Kendrick & Burges 1962, Ponge 1991). Sizeable bacterial populations were found to develop only at the more advanced stages of decay (Richards 1987, Ponge 1991). Thus the trophic structure of the nematode fauna of the litter horizon in this study (with BF:HF ratio's < 1) reflected the generally observed composition of the microflora of these horizons. The reported minor importance of bacteria in the initial stages of decomposition of pine needles are further supported by the occurrence of only two common bacterial feeding nematode taxa (*Panagrolaimus, Plectus*) in the litter horizons. The

occurrence of *Panagrolaimus* is possibly related to its opportunistic life strategy which may be advantageous in microhabitats with unpredictable feeding conditions (Chapter 5). However, life strategies of the species within the Plectidae are supposed to be less opportunistic (Schiemer 1983, Bongers 1990b). Three species of *Plectus* were found in this study, from which *P. acuminatus* and *P. pusillus* occurred in the surface horizons, and *P. longicaudatus* only beneath the litter horizon. Zell (1989) also found *P. acuminatus*, together with the opportunist *Rhabditis silvatica*, to be the first bacterial feeding colonizers of fallen beech leaves, whereas the surface horizons of another beech forest were dominated by *Plectus cirratus* (Volz 1951 *in* Twinn 1974). Twinn (1974) suggested that the large *Plectus* spp. of surface horizons, occupy a niche different from that of other bacterial feeding nematodes. This then should resolve such cases as the absence of the eurytopic bacterial feeding nematodes *Acrobeloides nanus, Wilsonema otophorum* and *Metateratocephalus crassidens* in the litter horizon. Schiemer (1983) hypothesized that the relatively long reproductive phase of *Plectus palustris*/*P. cirratus* can be advantageous in tiding over periods of food shortage. Moreover, the associated occurrence of some *Plectus* species with mosses and lichens (Nielsen 1949, 1967, Proctor 1984, Zullini & Peretti 1986, Bongers 1988) and their dominance in high latitude and altitude nematode communities (Procter 1984) indicate their ability to survive extreme climatological conditions, which is most pronounced in the litter.

The occurrence of soil fauna in surface horizons largely depends on the physiological tolerance of the species to fluctuations in microclimatological conditions (Anderson 1977, Rusek 1978, Chapter 5), such as fluctuating temperature and moisture conditions. The presence of water films is essential to the activity of nematodes. Thus species living in the surface horizons will exhibit relatively efficient water retention and greater ability to survive desiccation through anhydrobiosis. Because of the physical structure of the litter horizon, avoidance of desiccation by migration to deeper soil horizons is not expected. Because loss of water due to transpiration decreases exponentially for animals with smaller surface:volume ratio's (Vannier & Verhoef 1978), the occurrence of relatively large nematodes in the 0-10 cm mineral soil of the stages 1 and 2 and in the litter horizons (Table 6.3 and text), may reflect selection to reduce such water loss. However, as nematode movement depends on adequate water film thickness surrounding soil particles, and as water loss may induce nematodes to enter the state of anhydrobiosis (Demeure *et al.* 1979), the duration of activity of thicker nematodes like those found in the litter horizons, will be shorter than that of more slender specimens. If there is a trade-off between surface:volume ratio and body diameter it can be hypothesized that, body-length:body-width ratio's of nematode faunae of surface horizons will be large compared to nematode faunae of microhabitats with less extreme fluctuations in moisture conditions. Thus, in relation to nematode size (body length), body width of the nematode fauna of the surface horizons will be relatively small. Average body-

length:body-width ratio's of the nematode fauna of the soil horizons of the successional stages 2-8 ranged from 29.7-30.6, 26.3-29.2, 22.1-27.7 and 25.8-27.2 for the litter, fermentation and humus horizons and 0-10 cm mineral soil respectively, and was 30.4 for stage 1. Although these results support the hypothesis, it needs validation as body width was not measured directly but was calculated from longitudinal section area divided by body length.

Odum (1969) listed trends to be expected in the successional development of ecosystems. He expected populations of relatively small animals in the initial stages of succession, and increasing body sizes as succession proceeds. Our results seem to contradict this hypothesis, because the largest nematodes were found in the bare drift sands of stage 1. With succession, average body length of the nematode fauna of the 0-10 cm mineral soil decreased. Similar results were obtained by Wasilewska (1971) for the nematode fauna of a successional sere of *P. sylvestris* in Poland. The organic matter content of the 0-10 cm mineral soil increased during succession (Emmer *et al.* 1991) and will be related to altered soil micromorphology. Just as increased bulk density in the sequence litter, fermentation and humus horizon (Emmer *et al.* 1991) was related to a decrease in average nematode length, this increase in organic matter content of the mineral soil may have resulted in selection for overall smaller nematodes in the course of succession.

In general, the distribution of plant feeding nematodes appears closely related to the distribution of roots, and many studies showed positive correlations between numbers of plant feeding nematodes and primary production (see Yeates 1979 and 1987 for discussion). In the litter horizons plant feeding nematodes associated with higher plants were absent, which can be explained by the absence of roots of herbs and *P. sylvestris* in these horizons. Root biomass in the fermentation and humus horizons increased during succession, and was highest in the fermentation horizons (Van Berghem *et al.* 1986). This corresponds with an increase in absolute numbers of plant feeding and bacterial feeding nematodes m^{-2} in the fermentation horizons, and with increasing biomass of the total nematode fauna of these horizons. Moreover, annual production of the herb layer of Scots pine forests of 45, 75 and 120 years old at Hulshorster sand also increased (947 (33% of standing crop), 1783 (37%) to 3206 (24%) $kg.ha^{-1}.y^{-1}$ respectively) (Moszynska 1991). Our findings are also supported by observations from a succession of *P. sylvestris* in Poland, where an increase in abundance and biomass of bacterial, hyphal and plant feeding nematodes was observed in the succession (Wasilewska 1971).

Densities of plant feeding nematodes were low in 0-10 cm mineral soil compared to numbers found in the fermentation horizons, indicating lower food supply in this horizon. Although root biomass estimates from the study area (van Berghem *et al.* 1986) were

restricted to the organic horizons it is reasonable to expect such lower root biomass in the 0-10 cm mineral soil.

Besides quantitative differences in root biomass and density, differences in root quality (e.g. root anatomy, nutrition, exudates, growth pattern, mycorrhizas) will also effect plant feeding nematodes (Yeates 1987). The different vertical distribution patterns of epidermal/root hair feeding nematodes and endo- or ectoparasitic nematodes (respectively group e and a-d in Yeates *et al.* 1993a) in the forested stages probably relates to species-specific rooting patterns and morphometric characteristics of the nematodes.

In the organic horizons the plant feeding nematode fauna was composed predominantly of genera belonging to plant feeding group e (*Filenchus, Malenchus*) and nematodes belonging to plant feeding groups a-d only occurred incidently. The latter were mainly restricted to the mineral soil where they coexisted with the taxa from plant feeding group e. Similar nematode distribution patterns were found in a 67 years old Scots pine plantation (D63) located in the north-eastern part of the Netherlands, where plant feeding taxa belonging to groups c-d (*Rotylenchus, Tylenchorhynchus, Trichodorus, Paratrichodorus*) were restricted to the mineral soil, and group e taxa (*Filenchus, Malenchus*) occurred both in the mineral soil and organic horizons (De Goede, unpubl.). Also in a 15-20 years old Scots pine forest in Sweden, nematode taxa belonging to plant feeding groups c-d (*Rotylenchus, Paratylenchus*) and e (*Filenchus, Malenchus, Coslenchus*) reached highest abundances in the mineral soil and fermentation-humus horizons respectively (Magnusson 1983b).

As the occurrence of plant feeding nematodes depends on their hosts, the differences in distribution of group e and a-d plant feeding nematodes can probably be related to the rooting pattern of the plant species involved. In Scots pine plantations similar to Hulshorster sand, it was shown that the roots of the dominant herb species of the forested stages of the successional sere (*Deschampsia flexuosa, Empetrum nigrum, Vaccinium myrtillus*) mainly occurred in the organic horizons, and only few roots grew vertically into the mineral soil (Nabuurs 1991). Persson (1980) found roots of *P. sylvestris* concentrated below the organic horizons, in the upper part of the mineral soil. And moreover, largest turnover rates of roots of *P. sylvestris* and dwarf shrubs occurred in the mineral soil and organic horizons respectively (Persson 1980). Thus a greater part of the roots in the fermentation horizons probably belonged to plant species of the herb layer, whereas in the mineral soil the proportion of *P. sylvestris* roots was larger.

Deschampsia flexuosa was the dominant herb in the stages 5-7, and its roots have smaller diameter (diameter 0.1-0.5 mm; Nabuurs 1991) than roots of *P. sylvestris, E. nigrum* and *V. myrtillus*. Yeates (1986, 1987) indicated that stylet length of nematodes functionally affects feeding, and differences in root diameter will influence the length of stylet required to utilise the root resource. The epidermal and root hair feeding *Filenchus* and *Malenchus*

species from the organic horizons at Hulshorster sand, had shorter stylets than those nematode species restricted to the mineral soil (stylet lengths $\leq 10\mu m$ and $> 10\mu m$ respectively). Stylet length of *Aglenchus agricola* De Man 1881, also classified as epidermal and root hair feeder but mainly restricted to the mineral soil, is 13-16 μm (Sanwal & Loof 1967). Thus, these observations indicate that distribution of plant feeding nematodes could be related to interspecific differences in food resource utilization.

The functional relationship between the epidermal and root hair feeders and roots of grasses like *D. flexuosa* is further supported by the observations that in the absence of a herb layer (stage 3) no group e taxa were found in the 0-10 cm mineral soil, and that in stage 4, where coverage of the herb layer was $<50\%$, lower abundances of group e taxa were found than in the next stages with complete cover. In addition, experimental removal of the herb and organic layers from some parts of Scots pine plantation D63, did not effect the occurrence of the group a-d genera *Tylenchorhynchus* and *Rotylenchus* in the 0-10 cm mineral soil one year after treatment (De Goede, unpublished data). Although these species can feed on *P. sylvestris* roots, grasses are important to *Rotylenchus* and other plant feeding group a-d taxa from Scots pine forest as well (Magnusson 1983b).

The total abundance of plant feeding nematodes, in particular epidermal and root hair feeders, in the 0-10 cm mineral soil of stage 6 (Table 6.1) coincided with maximum growing conditions for *D. flexuosa* (Van Berghem *et al.* 1986, Fanta 1986, Moszynska 1991). Both the absolute and relative annual production of *D. flexuosa* (1660 $kg.ha^{-1}.y^{-1}$ and 39% of its standing crop respectively) were the highest recorded in the succession, and comprised 93% of total production of the herb layer. In the oldest Scots pine stage these figures were 1541 $kg.ha^{-1}.y^{-1}$, 26% and 48% respectively. Besides, the proportion of above ground carbon stock transferred from the herb layer to the organic horizon by seasonal shoot death (42% of total standing crop) almost doubled the estimates of younger and older forested stages (Moszynska 1991). The increased abundance of plant feeding nematodes in stage 6 thus probably reflects a positive correlation with primary production (Yeates 1987).

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GENERAL DISCUSSION

NEMATODES AND ECOLOGICAL SOIL CLASSIFICATION AND ASSESSMENT

An ecological classification of terrestrial and aquatic soils of the Netherlands can serve as a tool to assess the sensitivity of the soil ecosystem to environmental pollution and to predict the ecological efficacy of specific policy measures (Klijn *et al.* 1991, Gleichman-Verheijen *et al.* 1991, Sinnige *et al.* 1992). Such a nation-wide ecological soil classification system finds its basis in a "classification" or in a "typology" approach (definition *sensu* Verdonschot *et al.* 1992).

The "ecotope system" as developed at the Centrum voor Milieustudies Leiden (CML) (Stevers *et al.* 1987) is an example of the "classification" approach. An ecotope is defined as a site characterized by its homogenous vegetation, stage of succession and abiotic characteristics important to plant growth (Stevers *et al.* 1987). Based on a set of biotic and abiotic 'site-factors', any Dutch soil can be assigned to a defined class representing an ecotope. The biotic part of the ecotope system is primarily plant-based. However, to improve the indicative qualities of the system, an extension with taxonomic groups which are more sensitive to environmental pollution than plants (e.g. terrestrial and aquatic soil fauna) is necessary (Klijn *et al.* 1991, Sinnige *et al.* 1992).

Such extensive biotic and abiotic information is the basis of the scenario to achieve an ecological classification of soils as proposed by Gleichman-Verheijen *et al.* (1991). They indicated that an ecological description of soils should consist of a set of parameters representing each of eight groups of main abiotic and biotic 'compartments' of the soil ecosystem. Based on an inventory among representatives from the field of soil science, soil biology and soil policy, it was proposed to explore the feasibility of an ecological soil classification following a "typology" approach, describing a "Central-Concept" with "Pattern-Cards". Based on the set of biotic and abiotic parameters, soils are classified into groups from which typical representatives act as Central-Concept. Subsequently, each Central-Concept, together with an accompanying set of descriptions of the phases of its possible degradation (e.g. resulting from eutrophication, acidification, pollution), is defined as a set of Pattern-Cards (Gleichman-Verheijen *et al.* 1991).

In spite of fundamental differences between the "ecotope" and "Central-Concept/Pattern-Card" approaches, the present study indicates that inclusion of information derived from the composition of the nematode fauna can be valuable to both. A typology of soils based on the composition of the nematode fauna largely paralleled existing soil and vegetation typologies (Chapter 4). However, compared to e.g. the vegetation typology, the nematode based soil typology resulted in either more (Milio Fagetum, Luzulo Fagetum, Degraded Fago Quercetum) or less (Pineteae, etc.) detailed

types. Such bias between typologies based on different groups of biota has to be considered when developing an ecological soil typology or classification which will serve as a reference to a biological assessment system. In order to harmonize the biological assessment system and its reference, it seems advantageous to base the reference system as well as the assessment system on same group of organisms (Chapter 4, Bongers 1990a, Bongers & Schouten 1991).

RESOLUTION OF THE NEMATODE FAUNA

The analyses presented in Chapter 4 showed, when compared to e.g. vegetation, a high similarity between the nematode faunae of the Sample Groups of the afforested sandy soils. These results were supported by studies of e.g. Johnson *et al.* (1972), Wasilewska (1970) and Norton & Hofman (1974). The high similarities as found in the present study could have originated from i. a general deterioration of these poorly buffered sandy soils (Chapters 2 and 4), from ii. the sampling strategy (Chapter 6) and/or from iii. the taxa identification level.

In the present study, nematodes were basically identified to genus. Some genera were divided into morphological groups. Compared to an identification to species, this inevitably resulted in a reduction of information. Whether this also resulted in a reduction of the discriminating ability between different classes is debatable. Preliminary analyses on a selection of data presented in this study (Bongers *et al.*, 1989) showed that a similar classification was obtained, whether based on nematode species or on genera. On the other hand, as was shown by the distribution of several of the distinguished morphospecies (Chapter 4), sub-division of genera contributed to the classification. The contribution of a taxon to the classification is supposed to depend on its frequency of occurrence, and if the taxon is not a species, on the "functional homogeneity" of the taxon. Examples are the genus *Paratylenchus* and the family Criconematidae which were composed of many relatively rare species which would have been of low value to the classification if included at species level. Divided into several morphospecies which were determined by differences in stylet length and thus could be regarded as functional groups (Yeates 1986, 1987), they showed distinct distribution patterns within the classification (table 4.1). On the other hand, genera like *Filenchus, Acrobeloides, Plectus* and *Aphelenchoides* occurred in almost every soil sample and will thus not contribute to a classification when included at genus level. In the case of *Acrobeloides* no further subdivision was possible as only one species was involved (*A. nanus*). The other three genera were represented by several species, but species identification was often difficult: only juveniles present; mixed species population with indistinguishable juveniles; not

enough specimens for proper identification; or inappropriate identification keys. The lack of appropriate identification keys, which also applies to other taxa such as *Ditylenchus*, *Eudorylaimus* and *Rhabditis*, is a problem that was recognized decades ago (Nielsen 1949) and which is still not solved (Ferris & Ferris 1989, Bongers 1990a, Bernard 1992). This is a serious obstacle in ecological studies utilizing nematodes (Bernard 1992).

NEMATODES AS INDICATORS OF ENVIRONMENTAL CONDITION OF SOILS

As formulated by Begon *et al.* (1986), different kinds of organisms are not distributed at random amongst different kinds of environment. There is, however, a correspondence between the two. The present study showed relationships between the distribution of nematodes, and soil and vegetation characteristics, including (micro)climatological conditions (Chapters 4, 5 and 6) and the availability of food sources (Chapters 5 and 6). Moreover, species abundances and distributions were found to respond to changes in environmental conditions, whether the changes were the result of natural habitat development. (Chapter 5 and 6) or of various kinds of environmental disturbances (Chapters 2 and 3).

Interpretation of effects of environmental disturbances can be based either on the analyses of effects on nematode species or on the analyses of nematological indices. Both approaches were followed in Chapter 3. In order to compare different studies or to standardize the interpretation of effects in different environments, the use of indices has advantages over species analyses. The Maturity Index is an index based on the ecological requirements of nematodes and has proved to be sensitive to various kinds of disturbances (Chapter 2 and 3, Bongers 1990b, Bongers *et al.* 1991, Yeates *et al.* 1993b, Freckman & Ettema 1993, Ettema & Bongers 1993). Thus the Maturity Index can be a general gauge of the condition of the soil ecosystem (Bongers 1990b). However, the c-p distribution, which forms the basis of the Maturity Index, can be analyzed in more detail by using c-p triangles. This offers the possibility of identifying different types of disturbances (Fig. 2.4), and opens perspectives to the development of nematological indices which indicate dominant environmental stresses (Bongers & Schouten 1991). In fact, promising support for such selective indices was found in the application of a preliminary ranking of nematode taxa according to their sensitivity to dehydration (Chapter 6). However, application and further development of these indices, whether indicative for general or specific environmental disturbances, definitely requires research on causal relations between the occurrence of species and the environmental factors (Kuyper 1990), as well as an extension of the autecological knowledge of many of the nematode species.

NEMATODES AND THE RECOVERY OF HABITATS

Many examples are available in which changes in the composition of the nematode fauna indicated disturbances of the soil environment (see Chapter 1). Only a few studies (e.g. Kappers 1990, Kappers & Manger 1990, Yeates *et al.* 1991, Ettema & Bongers 1993) describe nematode community re-establishment after disturbances. Prediction of the effects resulting from e.g. political measures on the ecological functioning of soils and the monitoring of long-term recovery processes are important aims of an ecological soil assessment system. Here, also, the Maturity Index and c-p triangles appeared to be useful instruments (Chapter 2, Figs. 3.4a-b, Ettema & Bongers 1993). The sequential changes in c-p distribution during the recovery of the habitat following a disturbance seemed to depend on the type of disturbance (Fig. 2.4). In the case of the manuring experiment described by Ettema & Bongers (1993), the nematode fauna returned to a c-p distribution similar to that which was found before manuring. Similar findings were shown in Fig. 3.4a-b following treatment of a forest soil with urea. Both treatments showed relatively strong effects, appearing within a short period of time, and persisting for only months. This contrasts sharply with the results of the liming experiments.

On the other hand, situations will occur in which the initial situation cannot be regained or is unknown. In these situations a preferred direction of development can be chosen as reference (Verdonschot *et al.* 1992). Such preferred directions of development can be visualized by arrows in c-p triangles as indicated in Fig. 2.4. Similar patterns can be found when nematode communities develop, induced by changing natural conditions as was shown for the nematode fauna of a primary succession (Chapter 5). Invasion of Scots pine trees and the subsequent development of an organic horizon meant an irreversible "disturbance" (a decreasing dominance of taxa belonging to c-p 3-5) to the nematode fauna of the *Spergulo-Corynephoretum*. Despite significant differences in the nematode species composition of the Scots pine forest, the nematode fauna of the Scots pine forest developed a c-p value group distribution similar to what was found in the preceeding *Spergulo-Corynephoretum*.

The recovery process of disturbed soil ecosystems will largely depend on the ecological requirements and life history characteristics of the species involved. Re-establishment of populations of species which became extinct following a disturbance will first depend on their colonization capacity. This is probably an important problem in poorly buffered sandy soils within the Netherlands (Chapter 3, Chapter 4).

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CURRICULUM VITAE

* 14 juni 1959	Geboren te Uitgeest
* 1976	Eindexamen Mavo-4, Henricus Mavo te Castricum
* 1979	Eindexamen Atheneum B, Bonhoeffer College te Castricum
* 1982	Kandidaatsexamen Biologie B1 met wiskunde, Vrije Universiteit te Amsterdam
* 1986	Doctoraalexamen Biologie (cum laude), Vrije Universiteit te Amsterdam - Hoofdvak: dieroecologie en ecotoxicologie (VUA) - Bijvakken: Diersystematiek en Zoögeografie (VUA, RUG) en Milieukunde (IvM) - 1ste graads leraar biologie
* 1987 - 1991	Assistent in opleiding bij de vakgroep Nematologie, Landbouwuniversiteit te Wageningen
* 1990 - heden	Universitair docent bij het Biologisch Station Wijster "Centrum voor Bodemoecologie" van de Landbouwuniversiteit te Wageningen

APPENDIX 1

Nematode taxa composition (%) of the samples taken from April to May 1988 of a variety of Dutch terrestrial habitats.

The Site (1-51) and Plot (a-c) codes correspond with the codes used in Van den Berg *et al.* (1990; their Plot codes are 1-3 respectively), who presented soil chemical and physical characteristics of a selection of the samples, and who made a topographic description of the sites. Furthermore, Forest type (see Table 4.3 for explanation) and Sample Group (Chapter 4) are given. The forest type of some of the plots was revised with respect to Van den Berg *et al.* (1990). Nematode taxa which occurred in a sample but did not form part of the analysis are indicated with "+".

Site		1	1	1	23	23	23	24	24	2	2	2	39	39	40	40	50	50	50	22	22	22	25
Plot		a	b	c	a	b	c	b	c	a	b	c	a	b	a	b	a	b	c	a	b	c	a
Forest type		CP	CP	CP	CP	CP	CP	LP	LP	LP	LP	LP	LP	LP	EP	EP	EP	EP	EP	EP	EP	EP	EB
Sample Group		G	G	G	G	G	G	G	G	G	G	G	G	G	G	F	G	G	G	G	G	G	F
7	malenchu	0	1	.	2	.	.	25	1	1	.	.	8	6	16	5	1	1	1	3	1	6	.
9	filen A	6	8	14	.	6	4	.	13	.	.	.	5	8	15	16	13	9	12	.	2	12	16
10	filen B	3	17	14	28	15	25	6	.	11	13	16	4	.	.	16	24	40	17	6	2	.	4
11	filen C	.	.	2	.	.	.	4	3	.	.	.	.	.	.	7	.	.	3	.	.	.	.
13	psil ae	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.
14	cechhexa	1	.	1	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	6
20	dolichor	.	.	.	.	.	.	5	5	6	2	7	.	.	1	.	1	.	6	.	.	1	1
23	bitylenc	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
24	helicoty	.	.	.	.	.	.	.	.	.	1	4	7	1	.	.	.	.	.	.	.	1	.
27	pratylen	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	4	.	.	.	.
31	meloidog	.	.	.	.	.	.	.	.	.	10	.	.	.	.	.	.	.	.	.	.	.	.
33	crico B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.
34	crico C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	7
37	hemicy B	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
42	ditylen	.	.	1	.	.	3	.	.	3	1	1	1	4	1	6	.	1	1	1	2	1	.
44	nothotyl	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
45	pseudhal	.	.	1	10	9	.	5	6	.	.	.	.	2	.	4	.	4	3	3	.	2	.
51	aphechoi	32	21	9	20	47	16	7	10	16	18	31	12	11	8	6	13	10	9	21	28	10	6
53	seinura	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.	.	.
54	rhab ae	.	1	.	1	.	2	1	7	1	17	.	1	1	4	4	1	.	.	2	2	5	5
58	heteroce	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	2	3	.	.
59	acrbeles	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.
60	abeloide	5	2	3	5	2	2	19	20	14	1	6	5	15	2	4	8	9	5	1	17	7	6
61	cervidel	10	3	5	.	.	1	2	1	11	6	7	1	8	3	1	3	5	4	2	3	.	.
67	terarrnu	6	9	10	1	.	1	.	.	1	3	9	16	.	6	6	1	.	4	1	.	3	1
69	metatera	3	1	5	6	.	4	8	6	.	1	2	6	1	8	7	1	.	5	12	5	14	1
70	dipl ae	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.
71	pristion	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.
72	monhyste	3	1	1	.	.	4	.	4	6	.	.	.	2	.	2	.	.	1	2	2	5	2
74	plectu B	3	3	4	3	.	.	.	.	2	1	.	.	.	5	5	.	.	.	.	.	.	.
75	pleclstr	6	8	11	10	9	15	12	11	8	15	7	11	12	9	2	9	6	8	11	10	11	1
76	plectu C	1	1	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
77	wilsonem	3	3	2	2	8	20	.	2	2	.	1	4	2	5	.	5	2	1	25	6	3	13
84	domorgan	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.
88	micr	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
91	prismato	4	7	9	2	.	2	2	1	6	3	3	9	5	6	1	3	.	1	2	5	3	3
107	quds ae	1	2	1	4	2	1	.	.	3	.	1	5	9	3	2	1	1	6	3	1	5	2
117	tchmolai	.	.	.	1	.	.	.	.	.	.	1	.	.	1	2	.	.	4	.	.	.	1
119	tylolaim	.	4	9	3	.	.	.	1	1	4	.	3	2	6	2	10	9	5	1	6	7	28
120	trich ae	.	.	.	1	.	.	.	.	.	.	.	.	1	.	3	1	.	.	.	.	.	.
121	steinern	5	7	1	1	.	.	1	3	3	2	4	.	6	.	.	4	.	.	1	5	1	.
126	sporellu	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.

APPENDIX 1 *Continued*

Site	27	27	27	26	26	26	16	16	16	21	21	21	42	42	4	4	4	41	47	47	47	48	48	48	49	49	4
Plot	a	b	c	a	b	c	a	b	c	a	b	c	a	b	a	b	c	a	a	b	c	a	b	c	a	b	c
Forest type	CQ	CQ	CQ	CQ	CQ	CQ	CQ	CQ	CQ	BQ	BQ	BQ	BQ	BQ	BQ	BQ	BQ	BQ	FQd	FQd	FQd	FQd	FQd	FQd	FQd	FQd	FQ
Sample Group	F	F	F	G	F	F	F	F	F	E	F	F	F	F	F	F	F	F	F	D	D	F	F	F	E	F	
2 lelenchu	.	.	.	.	.	.	1	.	.	7	5	5	.	.	.	.	.	.	1	+	.	.	.	1	1	.	
6 aglenchu	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	
7 malenchu	.	1	+	43	5	26	2	4	4	12	12	22	1	7	1	1	.	.	.	.	1	.	.	.	.	.	
8 tylenchu	.	.	.	.	.	.	.	.	.	.	.	2	.	3	.	.	.	1	.	.	.	.	.	.	.	.	
9 filen A	11	5	4	.	2	6	14	8	16	15	17	15	27	36	10	15	20	17	24	10	4	10	8	6	12	3	
10 filen B	11	25	18	1	27	11	4	10	10	7	8	2	7	3	3	7	14	5	10	62	63	28	8	16	13	45	2
11 filen C	.	.	.	.	.	.	.	.	.	.	3	.	.	.	1	15	4	2	.	.	.	1	.	.	.	.	
14 cechhexa	9	4	1	.	.	2	16	7	5	1	8	11	4	6	11	5	2	11	2	+	7	3	13	4	16	2	
16 ecphtuin	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
18 merliniu	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
20 dolichor	.	.	1	.	.	.	.	.	.	.	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
24 helicoty	.	.	2	.	.	.	.	.	8	.	.	.	1	.	.	.	1	21	3	2	3	.	.	2	1	.	
25 rotylenc	1	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
26 rotychul	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
27 pratylen	.	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
29 hoplotyl	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	
33 crico B	.	.	.	.	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
34 crico C	1	1	1	.	.	.	.	4	.	.	1	1	7	.	6	.	2	1	8	.	5	3	5	4	4	5	1
35 hemicric	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	
37 hemicy B	.	.	.	.	.	.	.	.	.	7	6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
38 paraty A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	2	.	.	.	.	2	.	
40 paraty C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1	3	.	1	.	
42 ditylen	.	.	.	.	.	.	6	8	2	6	3	4	2	2	1	.	.	2	3	.	.	.	.	.	17	5	
45 pseudhal	.	.	.	.	1	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
47 dotylaph	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
48 aphechus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	
49 acid ae	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
51 aphechoi	2	6	4	3	8	3	9	5	6	15	5	4	2	2	7	1	3	3	1	1	4	.	6	5	4	3	
54 rhab ae	22	6	6	5	2	5	1	2	3	.	.	.	.	2	1	2	.	1	2	3	1	3	.	2	8	2	
58 heteroce	1	.	.	.	.	.	2	.	.	1	1	1	.	.	.	.	.	.	1	.	5	1	.	.	.	.	
59 acrbeles	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
60 abeloide	4	10	4	7	5	5	9	14	5	4	5	8	5	1	14	6	6	4	4	4	2	3	5	4	7	8	
61 cervidel	.	.	.	.	.	.	.	.	.	.	.	.	2	4	1	.	1	2	.	+	.	1	3	2	.	1	
63 drilocep	.	.	.	.	6	3	3	11	5	.	.	.	.	1	.	.	.	1	.	.	.	1	4	6	3	1	
67 terarrnu	1	4	16	.	8	1	4	3	5	1	1	.	4	.	19	6	6	8	9	.	.	1	8	6	1	7	
68 teradada	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
69 mstatera	8	3	12	8	4	1	2	3	5	4	4	3	2	1	4	13	2	3	2	.	.	3	1	4	2	4	
71 pristion	16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
72 monhyste	2	9	6	7	8	6	6	.	5	.	2	5	6	4	1	12	2	.	1	2	1	11	5	6	1	1	
74 plectu B	.	.	.	.	.	1	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	
75 plectu A	+	7	3	2	.	2	6	2	1	1	2	2	6	6	2	4	3	5	5	3	1	3	5	5	1	6	
77 wilsonem	1	4	3	7	11	14	8	10	11	1	3	5	3	3	1	1	2	.	1	+	.	2	2	5	2	1	
82 lept	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	
84 domorgan	.	.	.	1	.	.	1	.	.	.	.	1	.	4	2	1	.	.	.	2	1	2	.	.	.	.	
86 achrruri	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
87 pdescirc	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
91 prismato	4	8	3	5	6	7	1	1	.	3	5	2	2	5	.	1	4	3	9	3	+	4	8	3	.	1	
94 alaimus	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
96 paramp B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	
98 monon ae	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
105 pro/meso	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	
107 quds ae	.	3	4	2	1	1	.	1	.	2	6	.	6	3	.	6	11	3	.	2	2	7	3	13	1	4	
117 tchmolai	3	2	4	1	.	.	.	.	.	.	.	1	.	.	.	.	.	1	.	.	.	.	.	1	.	.	
119 tylolaim	2	+	4	.	3	.	5	4	4	2	1	1	9	3	8	5	10	2	2	2	1	4	2	3	1	2	
120 trich ae	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	
121 steinern	1	.	1	4	.	3	.	1	2	6	1	3	1	1	5	2	5	1	7	1	+	4	7	5	1	1	
125 nemato A	.	.	.	.	.	.	1	1	3	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.	.	

Site	41	31	31	31	19	19	19	34	34	33	14	14	14	45	45	45
Plot	b	a	b	c	a	b	c	a	b	a	a	b	c	a	b	c
Forest type	BQm	BQm	BQm	BQm	BQm	BQm	BQm	FQm	FQm	FQm	FQm	FQm	FQm	FQm	FQm	FQm
Sample Group	D	D	D	D	D	D	D	F	D	D	D	D	D	D	D	D
2 lelenchu	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+
7 malenchu	3	.	3	.	1	1	9	2	+	.	.	2	1	.	.	1
9 filen A	3	22	4	20	15	6	17	17	2	17	5	13	25	8	16	13
10 filen B	4	5	7	9	24	12	5	27	8	40	13	48	17	57	30	36
11 filen C	.	3	.	1	13	21	3	.	11	.	.	.	.	20	17	16
12 miculenc	.	.	.	.	1	.	1	.	.	.	.	.	.	.	.	.
14 cachhexa	1	.	2	9	1	.	4	3	3	5	6	2	1	2	7	4
16 ecphtuin	1	.	1	3	.	.	.	2	.	.	.	.	.	.	.	.
24 helicoty	44	25	3	.	5	1	8	.	1	1	.	1	.	.	.	.
25 rotylenc	.	1	.	.	.	.	.	1	.	.	.	.	.	.	.	1
27 pratylen	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
29 hoplotyl	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
33 crico B	.	.	.	.	.	.	.	1	3	.	.	.	.	.	.	.
34 crico C	4	4	24	5	2	4	1	1	23	2	5	5	5	.	4	.
36 hemicy A	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.
40 paraty C	.	2	8	1	.	.	.	1	4	1	1	.	3	.	3	1
42 ditylen	3	2	2	.	.	.	1	.	.	.	2	.	.	.	.	1
44 nothotyl	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
45 pseudhal	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.
51 aphechoi	12	14	10	30	11	31	7	4	4	7	7	6	18	2	.	7
52 laimaphe	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
53 seinura	.	.	.	.	.	.	.	.	.	4	.	.	.	.	.	.
54 rhab ae	1	1	13	.	1	.	.	4	+	2	2	3	1	+	1	2
56 cephalob	.	.	.	.	3	.	2	.	.	.	.	.	.	1	.	.
58 heteroce	.	.	2	3	3	.	8	1	1	1	.	.	1	.	.	.
60 abeloide	5	5	6	6	4	6	12	5	4	+	12	5	11	1	5	3
61 cervidel	.	.	.	.	.	1	.	2	.	.	.	+	.	.	.	.
63 drilocep	1	.	.	.	.	.	1	2	.	2	.	3	2	.	1	1
64 panagrol	.	.	.	.	.	.	.	.	.	.	32	.	.	.	.	.
66 teracost	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
67 terarrnu	.	2	1	.	.	.	.	.	1	.	.	.	.	.	2	.
68 teradada	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
69 metatera	1	.	.	.	.	.	2	7	11	3	1	1	.	.	1	.
72 monhyste	.	2	1	3	.	.	1	4	1	.	1	.	3	1	.	.
74 plectu B	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.
75 plectu A	1	4	3	8	.	.	1	4	3	.	1	1	1	2	1	4
76 plectu C	.	1	.	.	.	.	.	.	1	.	.	.	.	.	.	.
77 wilsonem	.	1	.	1	.	1	.	3	1	.	.	1	2	.	.	1
80 chgaboet	.	.	.	.	12	13	9	.	.	.	.	.	.	.	.	.
84 domorgan	.	3	.	.	.	.	1	.	1	1	.	.	+	.	2	.
91 prismato	5	4	1	1	3	1	.	3	2	2	2	1	2	5	2	3
94 alaimus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
96 paramp B	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
105 pro/meso	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
107 quds ae	1	.	4	.	1	1	3	.	4	.	1	1	1	+	1	.
117 tchmolai	8	.	.	.	.	.	.	.	1	.	.	.	.	.	2	.
119 tylolaim	2	1	2	.	.	.	.	6	4	10	7	6	6	+	4	4
120 trich ae	.	.	.	.	.	.	.	1	3	.	.	.	.	+	1	1
121 steinern	.	.	.	.	.	.	1	3	.	.	.	.	.	3	.	1
124 loofthie	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.
125 nemato A	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.
127 nemato C	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.

APPENDIX 1 *Continued*

Site	3	3	3	20	20	20	9	9	9	12	12	12	37	37	46	46	46
Plot	a	b	c	a	b	c	a	b	c	a	b	c	a	b	a	b	c
Forest type	FQ	FQ	FQ	FQ	FQ	FQ	FQ	FQ	FQ	FQ	FQ	FQ	FQ	FQ	FQ	FQ	FQ
Sample Group	F	F	F	F	F	F	E	E	E	E	E	E	E	E	E	E	E
2 lelenchu	.	2	1	.	1	3	.	.	2	.	.	.	.	.	.	2	1
7 malenchu	.	.	.	.	5	.	.	.	.	1	.	.	.	2	1	.	.
9 filen A	18	5	3	8	11	7	3	16	30	5	.	.	1	4	5	5	25
10 filen B	27	22	15	15	6	3	14	26	.	32	27	20	78	53	54	41	22
11 filen C	1	.	.	.	6	.	4	.	15	.	.	.	.	.	.	.	.
14 cechhexa	5	3	9	21	6	25	23	31	16	3	7	.	.	1	10	1	10
20 dolichor	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	1	.
24 helicoty	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	1
25 rotylenc	.	.	.	.	.	.	.	3	.	.	.	.	.	.	.	.	.
27 pratylen	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.
33 crico B	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.
34 crico C	5	12	2	.	.	.	.	5	1	.	15	.	.	1	.	.	.
35 hemicric	1	.	5	.	.	.	.	.	.	.	.	.	.	.	.	2	1
37 hemicy B	.	.	.	8	1	7	.	.	.	.	.	.	.	.	.	.	.
40 paraty C	.	.	.	.	.	.	.	.	.	1	2	2	.	.	.	.	3
42 ditylen	3	7	6	5	4	6	28	9	10	35	28	41	4	15	17	24	13
44 nothotyl	.	.	.	.	9	.	.	.	.	.	.	.	.	.	.	.	.
45 pseudhal	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.
51 aphechoi	2	5	1	1	6	2	4	2	17	5	9	7	4	2	5	1	5
54 rhab ae	.	.	1	2	.	8	1	.	.	.	.	1	.	2	.	4	1
58 heteroce	.	.	1	.	1	.	.	.	.	.	1	.	.	.	1	.	.
60 abeloide	2	3	4	9	1	10	11	2	3	5	2	8	5	5	1	7	6
61 cervidel	11	3	1	.	.	.	3	3	.	2	1	1	.	.	1	1	.
63 drilocep	.	2	.	1	.	1	.	+	.	2	.	.	.	1	.	.	1
67 terarrnu	2	4	6	3	9	4	.	.	1	.	.	.	.	.	.	1	.
69 metatera	1	4	9	5	7	5	1	.	.	1	1	2	1	3	1	.	.
70 dipl ae	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.
72 monhyste	.	2	3	3	2	1	.	.	.	1	.	.	.	1	.	1	1
74 plectu B	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
75 plectu A	2	1	2	7	2	4	2	.	4	1	.	4	2	2	.	1	.
77 wilsonem	4	4	3	4	10	4	.	.	1	.	.	1	+	2	+	1	.
84 domorgan	.	.	.	.	1	4	.	.	.	.	1	.	.	.	.	.	.
91 prismato	2	4	3	1	4	2	.	1	.	1	.	1	.	1	.	.	3
107 quds ae	3	10	4	.	1	2	.	.	.	.	.	.	1	3	3	.	1
119 tylolaim	3	4	3	5	2	1	4	1	.	4	3	9	.	.	1	4	4
120 trich ae	.	.	.	.	1	.	.	.	.	1	.	1	.	.	.	.	.
121 steinern	5	4	8	.	2	.	.	1	.	.	3	.	4	2	+	2	.
125 nemato A	5	1	9	1	.	3	.	.	.	.	.	.	.	.	.	3	1

Site	7	7	7	10	10	6	6	13	10	11	11	11	36	36	36	43	43	43	35	35	35	51	51
Plot	a	b	c	a	b	a	b	a	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b
Forest type	MF1	MF1	MF1	MF1	MF1	MF1	MF1	MF2	MF2	MF2	MF2	MF2	MF2	MF2	MF2	MF2	MF2	MF2	MF2	MF2	MF2	MF2	MF2
Sample Group	D	F	F	E	D	C	D	C	D	D	D	D	E	E	E	E	F	E	F	D	E	F	F
2 lelenchu	.	.	.	.	.	1	.	+	.	.	.	.	.	.	1	.	.	.	.	+	4	.	.
4 basiria	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.
7 malenchu	.	.	1	1	.	.	.	1	.	.	.	.	1	.	1	.	1	1	.	.	.	3	.
9 filen A	3	6	16	10	17	3	6	12	4	.	16	3	17	20	48	6	1	7	17	17	7	8	2
10 filen B	6	3	16	14	20	2	.	.	34	28	16	36	25	53	30	44	8	14	21	31	24	30	17
11 filen C	.	.	3	1	1	.	22	9	.	27	3	24	1	.	.	4	3	5	1	17	3	1	2
14 cechhexa	32	5	+	3	4	7	11	10	29	17	19	9	5	1	2	14	21	8	3	4	10	11	17
15 cechlept	.	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
16 ecphtuin	2	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.
24 helicoty	.	.	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
25 rotylenc	23	10	10	1	3	12	2	17	17	.	2	.	.	.	.	.	.	.	3	.	2	.	.
27 pratylen	.	.	.	.	1	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	1
29 hoplotyl	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	+	.	.	.	.	1	+
30 heterode	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	1	.	.	.	.
31 meloidog	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
32 crico A	.	.	.	.	.	.	.	1	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.
33 crico B	.	.	.	2	2	.	.	.	.	.	.	+	.	.	.	.	2	1	.	.	.	.	.
34 crico C	.	7	.	.	.	.	2	.	.	1	3	6	.	1	.	.	.	1	1	7	4	.	.
37 hemicy B	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
39 paraty B	.	.	.	12	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
40 paraty C	.	9	2	14	1	9	6	5	4	1	.	2	.	.	.	.	.	.	.	1	1	.	.
42 ditylen	1	.	1	7	.	4	3	6	1	2	1	2	21	3	9	8	10	18	12	3	11	+	5
44 nothotyl	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	4	.	.	.	.
45 pseudhal	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
47 dotylaph	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
48 aphechus	.	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
51 aphechoi	3	7	9	3	2	1	4	2	4	7	5	4	3	5	4	3	1	12	1	.	2	14	5
54 rhab ae	9	20	9	7	5	7	11	2	.	.	4	2	2	1	.	2	6	4	2	5	2	4	2
56 cephalob	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
57 eucephal	.	.	.	.	.	+	.	.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.
58 heteroce	1	.	.	.	.	.	+	.	.	1	1	.	.	.	.	.	.	.	2	.	.	2	.
60 abeloide	1	4	7	4	4	9	3	4	7	1	6	1	9	1	1	5	3	6	4	2	9	4	2
61 cervidel	.	.	.	.	1	.	.	1	1	.	.	.	.	.	.	.	5	.	5	2	4	1	1
63 drilocep	.	5	5	.	5	1	3	.	.	1	4	.	1	.	1	1	3	5	.	.	.	.	3
67 terarrnu	4	2	1	1	.	7	8	.	+	.	.	.	2	.	.	1	5	3	6	4	3	7	11
69 metatera	1	2	1	.	3	1	.	5	.	.	.	2	.	1	.	1	3	2	1	.	.	5	4
70 dipl ae	.	.	.	.	6	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
72 monhyste	1	.	6	3	1	2	2	.	.	.	.	1	1	.	.	.	3	.	6	.	.	1	2
74 plectu B	.	.	.	.	3	7	.	.	.	.	3	.	.	.	.	.	6	.	.	1	.	.	.
75 plectu A	1	7	4	.	.	3	5	3	.	5	6	3	.	.	.	2	.	2	3	2	4	2	1
76 plectu C	.	.	.	.	.	1	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
77 wilsonem	2	4	4	.	.	.	3	.	.	1	2	.	1	+	.	.	1	.	1	.	4	+	3
82 lept	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.
83 cylindro	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
84 domorgan	.	.	.	1	.	.	.	4	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
85 rhbdterr	2	.	.	.	.	15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
91 prismato	2	2	1	1	3	3	.	5	.	.	4	2	2	.	.	.	5	4	.	2	.	+	4
93 tripul A	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
94 alaimus	.	.	.	2	2	.	3	.	.	.	.	.	.	.	.	.	.	.	2	.	3	.	.
95 amphidel	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
96 paramp B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1	+
97 paramp A	1	2	.	3	5	5	3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
98 monon ae	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
107 quds ae	1	2	2	1	.	.	1	.	.	1	4	.	1	2	.	.	2	.	1	.	1	.	1
114 dmoimico	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.	.	.	.	.
118 diphther	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
119 tylolaim	.	2	.	.	1	.	2	1	+	.	.	1	4	4	1	.	2	7	2	1	.	5	7
120 trich ae	.	.	.	+	.	.	.	10	.	3	2	1	.	.	.	1	6	+	1	1	.	+	2
121 steinern	.	.	.	1	.	.	.	.	.	1	.	2	2	6	2	8	1	+	1	2	1	.	.
124 loofthie	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3
125 nemato A	.	2	3	.	.	.	1	.	.	2	.	.	2	1	1	.	.	.	.	.	1	.	.

APPENDIX 1 *Continued*

Site	8	8	8	5	5	5	34	29	30	17	17	17	38	38	28	28	28
Plot	a	b	c	a	b	c	c	a	a	a	b	c	a	b	a	b	c
Forest type	LF	LF	LF	LF	LF	LF	SC1	SC1	SC	SC	SC	SC	SC	SC	SC	SC	SC
Sample Group	D	F	F	F	D	D	F	F	F	D	D	D	D	C	C	C	C
2 lelenchu	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	6	.
6 aglenchu	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.	.	.
7 malenchu	2	.	2	.	.	1	.	.	.	+	1	6	1	7	2	.	2
8 tylenchu	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	.
9 filen A	17	21	4	43	25	33	9	5	4	.	7	7	.	33	26	12	17
10 filen B	8	9	3	21	33	28	30	2	46	+	3	2	25	10	20	9	7
11 filen C	15	7	4	2	7	16	.	.	3	74	44	41	42	2	9	.	2
12 miculenc	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
14 cephhexa	24	7	14	+	3	2	2	3	3	.	3	+	1	2	.	.	.
15 cephlept	.	.	.	.	.	.	.	10	.	.	1	.	.	.	1	.	5
16 ecphtuin	.	.	.	.	.	.	3	.	1	.	.	.	.	.	.	.	.
24 helicoty	.	.	.	.	.	.	2	.	.	.	.	1	.	.	+	.	.
25 rotylenc	1	8	2	.	.	.	3	.	.	2	3	1	.	1	4	4	1
27 pratylen	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.
30 heterode	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.
33 crico B	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1	.	.
34 crico C	.	.	3	+	5	2	3	.	1	9	18	23	.	2	2	6	2
39 paraty B	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.
40 paraty C	1	2	+	+	3	+	1	.	.	1	2	.	8	3	.	6	.
42 ditylen	.	1	.	.	.	1	.	1	.	2	1	.	1	.	1	2	7
44 nothotyl	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
46 iotospha	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	.
48 aphechus	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
50 aprutide	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	1
51 aphechoi	1	3	4	15	11	3	3	4	3	1	2	3	4	5	3	8	6
54 rhab ae	3	1	5	1	.	+	2	5	5	3	4	5	.	3	3	3	10
55 bunonema	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
56 cephalob	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	1	3
58 heteroce	.	.	.	.	.	.	.	.	.	.	+	.	.	3	1	1	2
60 abeloide	10	11	4	3	1	3	5	15	6	2	2	2	3	1	.	1	3
61 cervidel	.	.	1	+	+	.	1	6	6	.	.	.	.	.	.	.	.
63 drilocep	.	.	6	.	.	2	2	.	1	.	.	+	2	.	.	1	.
67 tararmu	.	5	9	4	+	1	6	6	2	+	.	1	1	1	2	1	2
69 metatera	2	1	3	.	.	.	5	10	2	+	+	2	.	.	1	6	2
70 dipl ae	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
72 monhyste	.	.	1	.	.	.	3	+	1	.	2	2	1	.	1	.	2
74 plectu B	+	.	.	.	.	.	.	+	.	.	2	.	2	6	.	3	4
75 plectu A	.	5	6	2	1	.	6	.	.	1	.	2	1	.	4	.	2
76 plectu C	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.
77 wilsonem	1	3	2	1	1	.	1	1	5	+	+	.	2	1	1	.	.
81 chgabeke	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
84 domorgan	.	1	.	.	.	.	2	1	.	.	.	.	2	.	.	2	2
85 rhbdterr	.	.	.	.	.	.	.	10	.	.	.	.	1	.	.	.	.
86 achrruri	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1
89 aulolaim	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+
90 bastiani	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	2
91 prismato	+	3	7	+	.	+	3	3	5	+	1	1	1	4	6	4	3
92 tripul B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.
94 alaimus	.	.	.	1	.	.	.	1	.	.	1	.	.	12	1	.	.
96 paramp B	.	.	.	.	.	.	.	.	.	+	.	.	.	.	1	1	1
97 paramp A	.	.	.	.	.	.	.	7	.	.	.	.	.	.	.	1	4
98 monon ae	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
101 nygolaim	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
102 nygeclav	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
105 pro/meso	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
107 quds ae	5	2	2	2	3	+	1	2	.	+	2	2	1	.	1	3	.
117 tchmolai	.	.	.	+	.	.	.	1	.	.	.	.	.	.	2	.	1
118 diphther	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	7	1
119 tylolaim	6	11	12	3	5	2	3	.	3	.	+	.	.	.	.	.	.
120 trich ae	.	.	.	.	.	.	.	3	.	+	.	.	.	.	2	.	2
121 steinern	.	.	1	2	.	.	5	.	2	.	+	.	.	.	.	1	.
125 nemato A	5	.	6	.	.	1	.	.	.	.	1	.	.	.	.	.	.
126 aporellu	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.	.
128 nemato B	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.

APPENDIX 2

Explanation of the abbreviation of the nematode taxa names as used in APPENDIX 1.

60	abeloide	Acrobeloides	7	malenchu	Malenchus
86	achrruri	Achromadora ruricola	31	meloidog	Meloidogyne
59	acrbeles	Acrobeles	18	merliniu	Merlinius
6	aglenchu	Aglenchus	69	metatera	Metateratocephalus
94	alaimus	Alaimus	12	miculenc	Miculenchus
95	amphidel	Amphidelus	72	monhyste	Monhystera
49	aoid ae	Aphelenchoidae	125	nemato A	Nematode A (like alaimid)
51	aphechoi	Aphelenchoides	128	nemato B	Nematode B
48	aphechus	Aphelenchus	127	nemato C	Nematode C
126	aporellu	Aporcelaimellus	44	nothotyl	Nothotylenchus
50	aprutide	Aprutides	101	nygolaim	Nygolaimus
89	aulolaim	Aulolaimus	102	nygeclav	Nygellus cf clavatus
4	basiria	Basiria	64	panagrol	Panagrolaimus
90	bastiani	Bastiania	97	paramp A	Paramphidelus A
55	bunonema	Bunonema	96	paramp B	Paramphidelus B
14	cechhexa	Cephalenchus hexalineatus	38	paraty A	Paratylenchus A
15	cechlept	Cephalenchus leptus	39	paraty B	Paratylenchus B
56	cephalob	Cephalobus	40	paraty C	Paratylenchus C
61	cervidel	Cervidellus	87	pdescirc	Prodesmodora circulata
81	chgabeke	Chronogaster cf "bekendelle"	75	plectu A	Plectus A
80	chgaboet	Chronogaster boettgeri	74	plectu B	Plectus B
98	monon ae	Mononchidae	76	plectu C	Plectus C
32	crico A	Criconematidae A	27	pratylen	Pratylenchus
33	crico B	Criconematidae B	91	prismato	Prismatolaimus
34	crico C	Criconematidae C	71	pristion	Pristionchus
83	cylindro	Cylindrolaimus	105	pro/meso	Pro/Mesodorylaimus
118	diphther	Diphtherophora	45	pseudhal	Pseudhalenchus
70	dipl ae	Diplogasteridae	107	quds ae	Qudsianematidae
42	ditylen	Ditylenchus	54	rhab ae	Rhabditidae
114	dmoimico	Dorylaimoides mycoletzkyi	85	rhbdterr	Rhabdolaimus terrestris
84	domorgan	Domorganus	26	rotychul	Rotylenchulus
47	dotylaph	Dotylaphus	25	rotylenc	Rotylenchus
63	drilocep	Drilocephalobus	53	seinura	Seinura
16	ecphtuin	Ecphyadophora tenuissima	121	steinern	Steinernema
57	eucephal	Eucephalobus	117	tchmolai	Tylencholaimus
9	filen A	Filenchus A	66	teracost	Teratocephalus costatus
10	filen B	Filenchus B	68	teradada	Teratocephalus dadayi
11	filen C	Filenchus C	67	terarrnu	T. terrestris/tenuis
24	helicoty	Helicotylenchus	120	trich ae	Trichodoridae
35	hemicric	Hemicriconemoides	93	tripul A	Tripula A
36	hemicy A	Hemicycliophora A	92	tripul B	Tripula B
37	hemicy B	Hemicycliophora B	20	dolichor	Tylenchorhynchus
58	heteroce	Heterocephalobus	8	tylenchu	Tylenchus
29	hoplotyl	Hoplotylus	119	tylolaim	Tylolaimophorus
46	iotospha	Iotonchidae/Sphaerulariidae	77	wilsonem	Wilsonema
52	laimaphe	Laimaphelenchus			
2	lelenchu	Lelenchus			
82	lept	Leptonchus			
124	loofthie	Loofia thienemanni			

Letter codes (A-C) in the nematode taxa names are explained in Materials & Methods of Chapter 4.

APPENDIX 3

Computerized construction of the equilateral c-p triangle

Construction of an equilateral c-p triangle can be done in a spreadsheet by means of the following formulae:

$$X = (100 - a) - (0.5 * b) \quad (1)$$

$$Y = ((-\sqrt{3}) * X) + ((100 - a) * \sqrt{3}) \quad (2)$$

X and Y are the co-ordinates of the sample in the triangle, with a is c-p group score 3-5 and b is c-p group score 1. The axes are chosen as in Fig. 2.1.