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Analysis of nematodes and soil-borne fungi from *Ammophila arenaria* (Marram grass) in Dutch coastal foredunes by multivariate techniques

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Abstract

A survey was carried out at nine locations in the Dutch coastal foredunes to identify the species of soil borne fungi and nematodes associated with *Ammophila arenaria* (Marram grass). *Ammophila arenaria* is a sand binding grass that is very important for the stabilization of coastal foredunes. Degeneration of the plants occurs at stabilized sites and is supposed to be caused by a combination of soil-borne fungi and nematodes. Canonical correspondence analysis (CCA) and two-way indicator species analysis (TWINSPAN) were used to examine which fungal and nematode species usually coexist in the rhizosphere of vigorous and early declining stands of *A. arenaria*. In total, 47 species of fungi and 10 genera of plant-parasitic nematodes were found. According to CCA, the community of soil organisms of stands that were more than 10 years old was significantly different from recently established stands of 3 years old. Also, the community of soil organisms isolated from calcareous locations differed significantly from that of lime-poor locations. No relationship between the vigour of the plants (vigorous vs. early declining) and the soil borne species composition was found, although in roots of vigorous stands, the number of nematodes was higher than that of early declining stands. A relatively large group of soil organisms occurred generally. This group possibly contains an ubiquitous pathocomplex that cause the growth reducing effects of biotic origin which generally occur in *A. arenaria*. Analysis of this group of nematodes and fungi by TWINSPAN resulted in 9 different combinations of concurring soil organisms of which 5 combinations were present at all investigated locations. Two of the latter combinations contained both nematodes and fungi. The first contained three endoparasitic nematodes (*Meloidogyne maritima, Heterodera* spp. and *Pratylenchus* sp.) that concurred with the fungus *Mucor hiemalis*. The second group contained *Heterodera* spp., *Telotylenchus ventralis, Filenchus* sp. together with the potentially plant-pathogenic fungi *Microdochium bolleyi* and Fusarium culmorum, as well as the fungi *Mortierella* sp. and *Trichoderma harzianum*, all in relatively high numbers.

It is concluded that both CCA and TWINSPAN are valuable exploratory techniques, especially when used in combination, to detect possible combinations of soil organisms which may be involved in the degeneration of *A. arenaria*. Further identifications of harmful organisms should be obtained from experiments.

Introduction

Soil-borne diseases appear to be common in vegetation succession of coastal foredunes. For instance, the nematodes *Longidorus dunensis* and *Tylenchorhynchus microphasmis* play an important role in the degeneration of *Hippophaë rhamnoides* (Sea Buckthorn), a shrub species that occurs in a

later successional stage in coastal foredunes [Maas et al., 1983; Zoon et al., 1993]. Such soil-borne diseases are supposed to have a high degree of specificity [Van der Putten et al., 1993]. It is, however, as yet unknown which soil organisms are involved in the decline of the different plant species that succeed each other.

Ammophila arenaria (L.) Link (Marram grass) is a sand binding grass that naturally dominates European coastal foredunes [Huiskes, 1979]. The vigour of *A. arenaria* is highest on sea-facing slopes where it is buried regularly by fresh wind-blown sand. When sand accumulation decreases, the plant degenerate [Huiskes, 1979; Willis, 1989]. The above-ground symptoms are a decline in flowering, a less dense vegetation with shorter culms and increasing amounts of dead shoots and leaves. The newly formed roots remain short, lack root hairs and are deformed [Eldred and Maun, 1982; Willis, 1989; Van der Putten et al., 1990]. In North-America and Canada, *Ammophila breviligulata* Fern., American Beachgrass, occurs under conditions comparable to those of *A. arenaria* in Europe. Both plant species grow vigorously in response to sand deposition and both plants degenerate when sand accumulation diminishes [Eldred and Maun, 1982; Maun and Baye, 1988; Baye, 1990].

Van der Putten et al. [1989] showed that degeneration of *A. arenaria* is strongly correlated with the presence of harmful soil organisms in its root zone. The effects of these soil-organisms were already presented in vigorous *A. arenaria* stands. Upward growth upon sand accumulation is supposed to allow the plants to escape from harmful soil organisms. This upward growth has to happen each year, as newly formed roots are colonized by the soil organisms within one growing season [Van der Putten et al., 1989].

A recent study showed that several nematode species may be involved in the decline of *A. breviligulata* [Seliskar and Huettel, 1993]. As with *A. arenaria* [Van der Putten and Troelstra, 1990], only low and variable numbers of nematodes were present in *A. breviligulata*'s root zone. Soil treatments with nematicides enhanced the growth of *A. arenaria* seedlings in spite of the low numbers of nematodes that were present. Soil treatments with the fungicide Benomyl also resulted in increased plant growth, but this may have been due to a nematicidal effect of the fungicide applied. Nevertheless, it was concluded that, apart from nematodes, fungi may also be involved in the degeneration of *A. arenaria* [Van der Putten et al., 1990].

In the present study, the composition of the communities of soil organisms (nematodes and fungi) in the rhizosphere of vigorous and early declining *A. arenaria* stands was investigated. Furthermore, the community of soil organisms of *A. arenaria*, previously planted in 'virgin' dredged sea sand, was compared to that of established dunes. Samples were taken from May till September to study potential changes in the community of soil organisms during the growing season. The concurrence of fungi and nematodes and the degeneration of *A. arenaria* was analysed using ordination techniques (Canonical Correspondence Analysis; CCA) and clustering (Two-Way INdicator Species ANalysis; TWINSPAN). The combinations of soil-borne fungi and (plant parasitic) nematodes thus generated were used to compare calcareous versus lime-poor foredunes and recently established versus existing stands of *A. arenaria*. It is discussed how these results may be used for further studies on the identification of the species involved in the degeneration of *A. arenaria*.

Materials and methods

Study sites

Samples were collected from vigorous and early declining stands of *A. arenaria* at nine locations along the Dutch coastal foredunes: Voorne (5 locations), Goeree and Schouwen, which are located in the calcareous dune area in the southern part of the Netherlands, and Callantsoog and Texel, located in the north western lime-poor area [Rozema et al., 1985] (Table 1). In 1986, the locations 1, 2, 3 and 5 had been raised artificially with dredged sea sand that did not contain the specific rhizosphere organisms of *A. arenaria* [Van der Putten, 1990]. Early 1987, the sand depot was reshaped and stands of *A. arenaria* were established upon culms (location 1), rhizomes (location 2), seeds (location 3) or a combination of culms and rhizomes (location 5). The different methods

Table 1. Characteristics of the locations used to study nematodes and fungi possibly involved in the degeneration of *A. arenaria*. The vegetation of *A. arenaria* was established 3 years ago starting from culms, rhizomes, seeds or culms and rhizomes or was a vegetation that existed for more than 10 years. Lime-content was high when the $CaCO_3$ content was up to 10% and low when less than 0.01% (Rozema et al., 1985). NL = Northern latitude, EL = Eastern longitude. At each location, samples were taken from vigorous and early declining stands

Location	Sample-numbers	Establishment	Lime-content	Coordinates (NL*EL)
1 Voorne	1, 2	recent, culms	high	51°52′ 4°04′
2 Voorne	3, 4	recent, rhizomes	high	idem
3 Voorne	5, 6	recent, seeds	high	idem
4 Voorne	7, 8	existing vegetation	high	idem
5 Voorne		recent, culms + rhizomes	high	idem
6 Goeree	9, 10	existing vegetation	high	51°45′ 3°50′
7 Schouwen	11, 12	existing vegetation	high	51°35′ 3°32′
8 Callantsoog	13, 14	existing vegetation	low	52°50′ 4°42′
9 Texel	15, 16	existing vegetation	low	53°07′ 4°45′

are described by Van der Putten [1990]. Voorne location 4 is a foredune that existed already for more than 10 years. At all the other locations, the examined stands were more than 10 years old and were regarded as naturally developing foredunes (Table 1). Vigorous *A. arenaria* stands were subject to annual burial by 10 to 30 cm of windblown sand from the beach. Early declining stands were those stands where sand accumulation was less than 5 cm at least during the last year.

General survey

In March 1990, soil samples were collected from the foredunes of Voorne (locations 1 to 4; Table 1), Goeree, Schouwen, Callantsoog and Texel (Table 1). At each location, two sites of 250 m long and 10 m wide (parallel with the coastline) were chosen: one in vigorous and one in early declining stands of *A. arenaria*. At each site, 12 random samples with a total of 20 kg were collected with a small shovel. The samples were taken close to tussocks of *A. arenaria* from the layer of sand between 5 to 40 cm below the sand surface containing roots. Per site, the 12 samples were sieved through a sieve with a mesh size of 1.5 cm and mixed gently. A subsample of 500 ml of soil, as well as 20 g roots were taken from each composit sample for the analysis of nematodes and fungi.

Seasonal and within-location variation

Seasonal sampling was conducted in May, June and September 1990 at Voorne-locations 3, 4 and 5 (Table 1). For logistic reasons, only vigorous stands of *A. arenaria* were sampled. Four composit samples, each made up of 12 random samples, were collected from each location in order to study the variation throughout the season. For the analysis of within-location variation only the samples of May were considered. Each of the 4 composit samples was sieved and homogenized. Of each composit sample, both roots (±20 g) and soil (±500 ml) were used for identification and quantification of fungi and nematodes.

Soil organisms

Free-living nematodes were isolated from a subsample of 300 ml by elutration [Oostenbrink, 1960]. Endoparasitic nematodes were isolated by cutting the collected roots into 1 cm pieces and extracting these in Baermann-funnels ['s Jacob and Van Bezooijen, 1984]. Subsequently, the nematodes were counted and identified to at least genus level according to Bongers [1988].

Soil-fungi were isolated by planting soil dilutions (10^{-2}–10^{-5}) in triplicate on malt extract agar (20 g malt extract (Oxoid), 3 g peptone (Oxoid), 15 g agar (Merck), 100 ppm validamycine (Solacol, Aagrunol [Gams and Van Laar, 1982]) and 50 ppm oxytetracyclin. Root-fungi were isolated from 0.5 cm pieces of root. Per

sample, 15 root pieces were washed three times in sterile demi-water and placed on malt extract agar. After incubating at 23 °C for 4 to 7 days, the fungi were subcultured on potato dextrose agar (Oxoid), counted and identified. Nomenclature according to Domsch et al. [1980] was used for the fungi other than *Fusarium* throughout this study. *Fusarium* species were identified according to Nelson et al. [1983].

Statistical analysis

All isolations from roots and soil of fungi and nematodes were analyzed by means of two-way indicator species analysis (TWINSPAN) [Hill, 1979] and canonical correspondence analysis (CCA). Both multivariate techniques are described in detail by Jongman et al. [1987]. After identifying the species and assessing their densities, numbers of fungi and nematodes were standardized by dividing the number of a species in a sample (n_i) by the largest number (n_{max}) of that species found in any of the samples according to $100 * n_i/n_{max}$ [Jongman et al., 1987]. By this standardization the data set is liberated from variation caused by the intrinsic differences between species of different taxonomical and functional groups, without introducing negative numbers, since they are not allowed for CCA and TWINSPAN.

With CCA, the samples are arranged according to species composition with environment as constraint in a canonical optimization process (direct gradient analysis). Forcing the theoretical variables to be linear combinations of environmental variables, relative numbers of fungi and nematodes can be related to these variables. In the ordination diagram, samples were similar soil-inhabiting species are close to each other, whereas samples with a different assemblages of soil organisms are far apart. The analyses were performed using the CANOCO programme developed by Ter Braak [1988]. The standardized data were sqrt-transformed to obtain non-skewed distributions. The environmental variables used (presented as zeros and ones) were derived from Table 1:

recently established (RE) = 1 versus
existing vegetation (EV) = 0
calcareous (LIM+) = 1 versus
lime-poor (LIM-) = 0
vigorous stands (VIG+) = 1 versus
early declining (VIG-) = 0

Relationships between the composition of the community of soil organisms and environmental data was investigated by using forward selection of environmental variables in CCA. Environmental variables were added as long as the significance level of the Monte Carlo permutation test of the eigenvalue of the first four canonical axes was below 0.05 [Ter Braak, 1988].

By TWINSPAN, a two-step analysis of the data is made. First the samples are clustered on the basis of their species composition. To account for differences in abundance of the species, pseudospecies are defined, based on so-called cut-levels for the abundances. We applied cut-levels 0, 5, 10, 20 and 50 to the standardized data ($100 * n_i/n_{max}$), resulting in a maximum of 5 pseudospecies per species. Secondly, the species are grouped based on their steadfastness to sample clusters. The samples within each cluster have a comparable combination of soil organisms and can be characterized by the occurrence of species clusters. The TWINSPAN analysis in this study was performed by making a dichotomy of 4 levels without using indicator species [Jongman et al., 1987], because we were not interested in the dominance of species within the clusters. The standardized data ($100 * n_i/n_{max}$) of the general survey and the sampling of May were analysed with the 5 cut-levels as presented above.

The data of the seasonal sampling were analyzed with TWINSPAN as presence/absence data. The latter was chosen instead of the standardized data due to infections of bacteria in the isolations of fungi. Calculations with numbers of fungi would, therefore, not be reliable. Divisions were made when the Eigenvalue was ≥ 0.25.

Results

General survey

In total, 34 species of fungi were isolated from the various samples in the general survey (Table 2). Thirteen additional species were found in the seasonal sampling. The genus *Penicillium* was not split-up into species. The genus *Phoma* was represented by *P. exigua* and *P. leveillei*. There seems to be a higher amount of CFU's of fungi (especially *Penicillium*) in samples from existing vegetation compared to those from recently estab-

Table 2. Colony forming units (CFU) per g dry soil of fungi isolated from soil (average of 3 replicates per sample) and from roots (number of colonies growing from 15 root pieces). The fungi were isolated from the root zone of vigorous (VIG+) and early declining (VIG–) stands of *A. arenaria* on calcareous or lime-poor foredunes. The *A. arenaria* stands which were sampled, were established 3 years ago (recent) or more than 10 years existing. Saprophagous fungi and pathogenic fungi are listed separately according to Domsch et al. (1980)

	Calcareous				Lime-poor	
	Recent		Existing		Existing	
	VIG+	VIG–	VIG+	VIG–	VIG+	VIG–
SOIL						
Saprophagous fungi						
Absidia corymbifera	0	0	0	110	0	20
Acremonium furcatum	70	0	0	0	0	0
A. murorum	0	0	0	0	0	170
A. rutilum	0	10	0	0	0	0
A. strictum	60	0	0	0	0	0
Apiospora montagnei	70	0	0	560	0	330
Aspergillus sydowii	0	0	110	0	0	0
Chrysosporum pannorum	0	0	110	0	0	0
Humicola grisea	0	10	0	0	0	0
Mortierella alpina	0	170	1910	110	0	0
Mucor hiemalis	0	80	1690	0	20	30
Nectria inventa	0	0	0	0	0	330
Penicillium spp.	170	180	1690	14560	10670	14670
Pleospora sp.	0	0	0	330	0	0
Stachybotrys chartarum	0	60	0	110	0	0
Scopulariopsis sp.	30	90	0	0	0	0
Trichoderma harzianum	130	0	110	670	20	1680
Trichothecium roseum	0	0	10	0	0	0
Verticillium lecanii	10	0	0	0	1330	0
Pathogenic fungi						
Alternaria alternata	10	0	0	0	0	0
Arthirinium phaeospermum	0	0	0	0	330	330
Chaetomidium fimeti	10	100	440	220	3670	830
Chaetomium funicola	0	0	0	0	170	0
C. globosum	0	0	0	0	330	0
Fusarium culmorum	20	120	20	330	170	80
F. nivale	0	0	1110	1110	330	0
Harzia acremonioides	30	0	0	0	0	0
Microdochium bolleyi	0	0	110	0	0	0
Phoma spp.	30	0	220	1780	0	0
Pyrenochaeta sp.	90	0	890	440	0	330
Ulocladium sp.	0	0	0	110	0	330
Total	730	820	8420	20440	17040	19130
ROOTS						
Saprophagous fungi						
Acremonium furcatum	0.7	0	0	0.3	0	0
Apiospora montagnei	0	0	0	0.7	5.5	0
Mortiertella alpina	+	+	+	+	+	+
Mucor hiemalis	3.0	5.3	0.3	0	0	0
Penicillium spp.	6.3	3.7	2.3	11.3	6.5	7.5
Plectosphearella cucumerina	0	0	0	0.3	0	0
Trichoderma harzianum	4.7	0.7	4.0	6.0	0	1.0
Pathogenic fungi						
Chaetomidium fimeti	0.7	0	0	0	0	0
Chaetomium globosum	0	0	0	0	1.0	2.5
Cladosporium cladosporioides	0.7	0	0.7	0	0	0
Fusarium culmorum	0	1.0	0.7	0	0.5	0.5
F. chlamydosporium	0.0	0	0	0.3	0	0
F. equiseti	0.3	5.0	0	0	0	0
Microdochium bolleyi	2.3	5.7	0.3	0.7	2.5	1.0
Phoma spp.	0	0	2.0	0.3	0	0
Ulocladium sp.	0.3	0	1.0	1.7	0	2.5
Mean number of colonies per 0.5 cm root	*1.3*	*1.4*	*0.8*	*1.4*	*1.1*	*1.0*

lished vegetation (Table 2). However, the number of colonies per 15 root species (Table 2) appears to be rather independent of the age of the vegetation.

Ten genera of nematodes were isolated (Table 3). Saprobiotic nematodes consisted of Rhabditida (e.g. *Acrobeles* sp.) and Dorylaimida (e.g. *Eudorylaimus* sp., *Aporcelaimellus* sp., *Mylonchulus* sp.). Approximately 30 plant parasitic nematodes per 100 ml of soil (bulk density = 1.4 g/cm^3) were extracted from each sample. In existing stands, higher numbers of plant parasitic nematodes were collected from roots of vigorous *A. arenaria* (about 20 per g fresh root) than from early declining stands (about 6 per g fresh root). This difference was not found in recently established stands.

Statistical analysis of the number of fungal CFU and the number of nematodes in soil could not be done due to the large variation and the relatively low number of samples.

The canonical correspondence analysis revealed some differences in species composition (Fig. 1). Three groups of species could be distinguished which were clearly related to the environmental variables (Fig. 1). A clear contrast was found between the community composition in calcareous soils and that at locations poor on lime, as is indicated by the centroids called LIM+ and LIM–, respectively. Recently established stands (RE) clearly differed from the existing stands (EV) in their rhizosphere species. Species groups related to these contrasts are indicated in Fig. 1 and listed in Table 4. Both contrasts were significant at the 0.05 level in the Monte-Carlo permutation test. In addition to the contributions of the other variables, the vigour of the various *A. arenaria* stands contributed little to the fit of the species data, as is also indicated by the short distance between the centroids of vigorous (VIG+) and non-vigorous (VIG–) stands (Fig. 1).

A group of 15 species of fungi and 10 genera of nematodes (excluding the non-plant-feeding nematodes and the Dorylaimids) occurred at all location independent of the environmental factors. These species are represented by symbols in the

Table 3. Nematodes isolated from soil (number per 100 ml soil) and from roots (number per g fresh roots). The nematodes were isolated from the root zone of vigorous (VIG+) and early declining (VIG–) stands of *A. arenaria* at calcareous and lime-poor foredunes. The *A. arenaria* stands which were sampled, were established 3 years ago (recent) or more than 10 years

	Calcareous				Lime-poor	
	Recent		Existing		Existing	
	VIG+	VIG-	VIG+	VIG-	VIG+	VIG-
SOIL						
Pratylenchus sp.	3.3	3.9	4.1	0.0	10.7	1.5
Paratylenchus sp.	2.1	3.1	1.0	1.0	1.5	0
Rotylenchus goodeyi	0	0	0	0	3.1	1.5
Helicotylenchus sp.	0	0	0	0	0	4.6
Filenchus sp.	2.9	5.1	10.2	11.2	7.6	6.1
Heterodera larvae	2.1	3.6	6.1	1.0	0	0
Meloidogyne larvae	3.9	4.5	14.3	4.1	0	1.5
Heteroderidae males	0	0.1	2.0	0	0	1.5
Aphelenchus sp.	1.3	0.5	1.0	2.0	0	0
Telotylenchus ventralis	9.6	12.0	4.1	3.1	0	0
Total plant parasitic nem.	25.2	32.8	42.8	22.4	22.9	16.7
saprobiotic nematodes	239.6	362.7	394.3	354.5	414.2	507.4
ROOTS						
Pratylenchus sp.	0.25	0.76	4.32	0.42	12.68	0.85
Paratylenchus sp.	0.29	0.31	0.59	0.33	0.51	0
Rotylenchus goodeyi	0.07	0	0.07	0.39	0	0
Helicotylenchus sp.	0	0	0.17	0.07	0.18	0
Filenchus sp.	1.11	1.38	1.06	0.75	3.33	2.29
Heterodera larvae	1.03	0.20	4.53	0.13	0	2.84
Meloidogyne larvae	0.63	0.20	16.05	0.46	2.02	1.69
Heteroderidae males	0.23	0	0.46	0	0.37	0
Aphelenchus sp.	0.13	0.34	0.07	0.57	0	0
Telotylenchus ventralis	1.17	1.67	0.57	1.32	1.10	0.85
Total plant parasitic nem.	4.91	4.86	27.89	4.44	20.19	8.52
saprobiotic nematodes	50.2	61.0	110.8	164.6	287.8	125.9

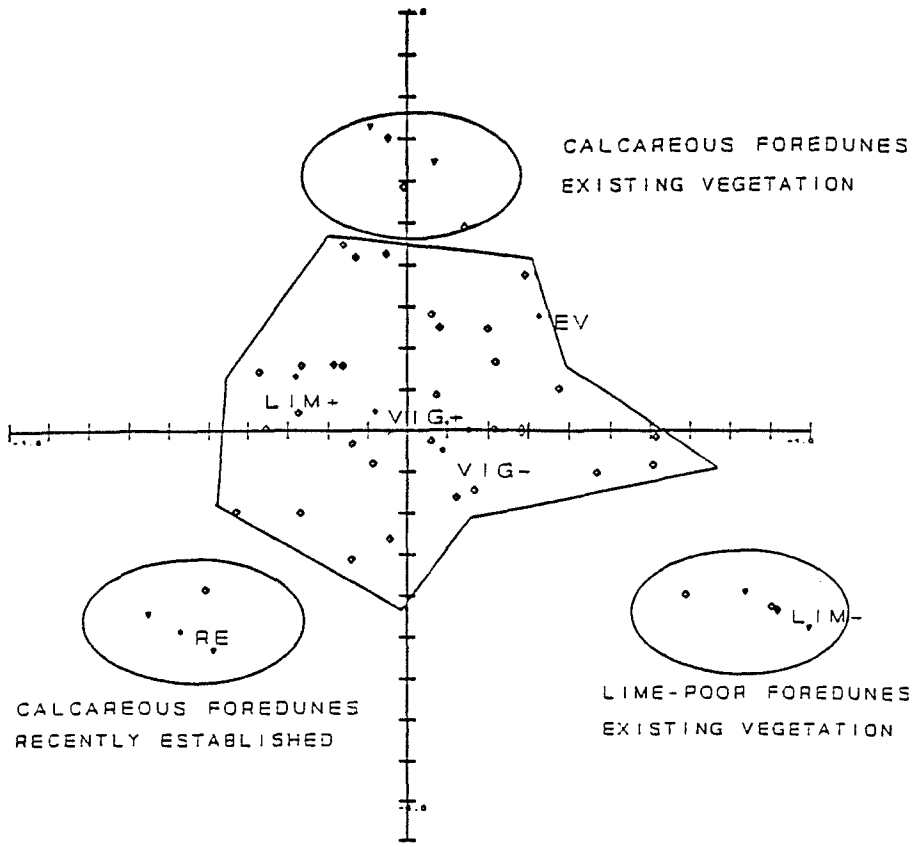

Fig. 1. The arrangement of species and environmental variables as generated by canonical correspondence analysis. The samples were collected from the root zones of vigorous (VIG+) and early declining (VIG–) *A. arenaria* stands of calcareous (LIM+) or lime-poor (LIM–) foredunes. The vegetation was recently established (RE) or already existing for more than 10 years (EV). Each dot represents one or two species. The 4 different assemblages of soil organisms are separated. The individual species from the 3 separate groups are presented in Table 4.

enclosure around the origin (Fig. 1). In total, 36.2% of the variation was explained by the four axes calculated in CCA. The first two axes, related to lime-content and age of the foredune, explained 10.9 and 7.1% of the variation, respectively. The remaining axes 3 and 4 enlarged the distance between the centroids of vigorous (VIG+) and non-vigorous (VIG–) stands, but did not result in a further separation of soil organisms within the central cluster.

When the same data were analyzed by TWINSPAN, 5 clusters of samples occurred when the threshold Eigenvalue was set at 0.25 (Fig. 2). Unlike CCA, TWINSPAN did not separate samples from the calcareous foredunes from those of lime-poor samples. Also samples taken from an existing vegetation were not separated from those from a recently established vegetation. In accordance with the CCA results, no divisions were made between samples of vigorous or early declining stands of *A. arenaria*. Neither did various types of planting methods result in a division between the sampled locations of Voorne (Fig. 2).

According to the created clusters of samples (Fig. 2), the soil organisms were assembled into 6 clusters (Fig. 3). A relatively large group of 18 species of fungi and 5 genera of nematodes isolated from soil, and 10 species of fungi and 8 genera of nematodes isolated from roots (r) (cluster 1; Fig. 3) were separated from the other soil organisms. The species of cluster 1 occurred in most soil and root samples. Most of these soil organisms were grouped in the centre of the axes

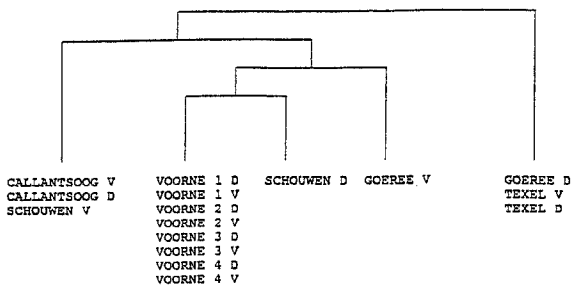

Fig. 2. Dendrogram of the locations. The divisions were made with the aid of TWINSPAN when Eigenvalue ≥ 0.25. Locations with the largest differences in numbers of species are split off first. V = vigorous and D = early declining stands, Voorne 1, 2, 3 and 4 represent the samples locations of Voorne with a vegetation recently established from culms (1), rhizomes (2) or seeds (3) or a vegetation existing for more than 10 years (4). Voorne, Goeree and Schouwen are located in the calcareous area, Texel and Callantsoog in the lime-poor foredunes (Table 1).

according to CCA (Fig. 1). In TWINSPAN, however, no correlation between the grouping of samples with similar environmental parameters and the subsequent grouping of species was found.

The more or less specific groups of soil organisms which were generated by CCA (Table 4) were henceforth omitted from the data set. The remaining group in the centre of the axes (Fig. 1) was subsequently analyzed by TWINSPAN. In total, 9 clusters were formed (Table 5). The first 4 clusters consisted of plant parasitic nematodes and fungi. Cluster 2 contained three endoparasitic nematodes: *Heterodera* spp., *Meloidogyne maritima*, and *Pratylenchus* sp. together with the fungus *Mucor hiemalis*. The organisms in cluster 4 occurred at almost all locations and often in relatively high numbers. In the cluster the plant-parasitic nematodes *Heterodera* spp., *Telotylenchus ventralis* and *Filenchus* sp. were combined with the potentially plant-pathogenic fungi *Fusarium culmorum* and *Microdochium bolleyi*, as well as the fungi *Mortierella* sp. and *Trichoderma harzianum*. Furthermore, 4 clusters were formed consisting only of fungi of which two (clusters 5 and 7) occurred at all locations (Table 5). Cluster 9 only contained the root/fungus feeding nematode *Filenchus* sp. and non-plant-feeding nematodes.

Seasonal and within-location variation

Almost all soil organisms isolated by sampling throughout the season were also found in the general survey. A slight shift from May to June and September was observed, which was, however, not significant (data not presented). The fungi *Phoma* spp., *Trichoderma harzianum, Fusarium culmorum* and the nematodes, *Hemicriconemoides* sp., Dorylaimidae together with the saprobiotic nematodes were isolated throughout the season.

The analysis with TWINSPAN of the samples collected in May at Voorne locations 3, 4 and 5, calculated with the abundance of species, revealed that the communities of soil organisms from the location with an existing vegetation differed from those that were recently established (Table 6). The organisms were arranged into 7 clusters. In the first cluster, soil organisms were grouped that mostly occurred in samples from recently established stands. Cluster 2 contained organisms that were present in relatively high numbers at almost all investigated locations. Clusters 5, 6 and 7 contained soil organisms specific to location 4 (existing vegetation). In recently established stands originating from seeds, the community of soil organisms was similar to those in stands grown from culms and rhizomes. *Microdochium bolleyi* and the non-plant-feeding nematodes were found in each of the samples. *Penicillium* spp. were abundant in samples of site 4 (Table 6).

An analysis by TWINSPAN of the soil organisms of the samples collected in May showed that the replicates were generally grouped into the same cluster, indicating that the within-location variation was small, whereas the between-location variation was large (Table 6).

Discussion

In this survey, 47 species of fungi and 10 genera of plant parasitic nematodes were isolated from the root zone of *A. arenaria* (Tables 2, 3 and 6). Among the fungi 18 plant pathogens, 2 pathogens of seeds (*Chaetomium* spp.) and 2 secondary pathogens were found, whereas the remaining 25 species have been described as saprophytes [Domsch et al., 1980]. A large number of these species of fungi was also isolated from the root

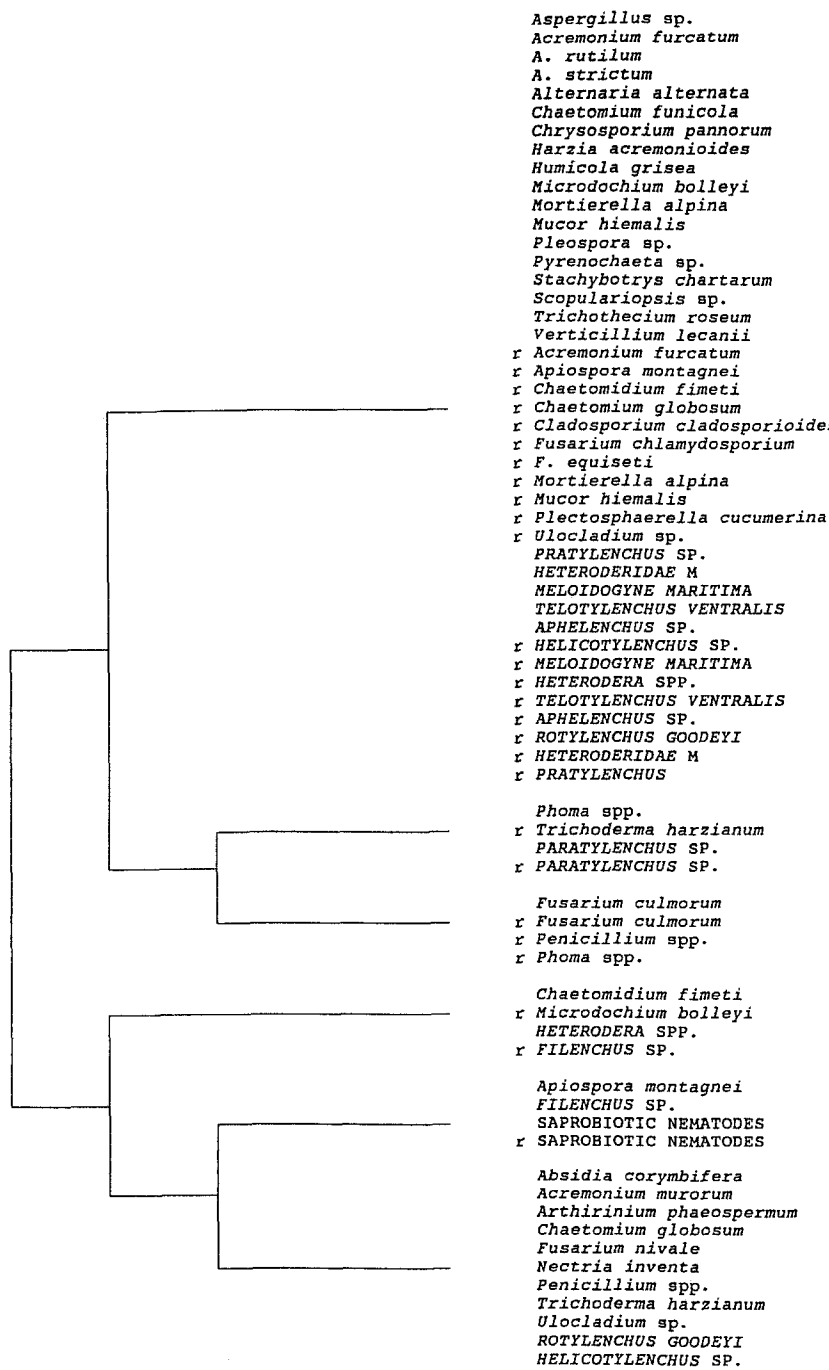

Fig. 3. Dendrogram of the nematode and fungal species from the root zone of *A. arenaria*. The divisions, based on the groups of samples according to locations, were made by TWINSPAN when Eigenvalue ≥ 0.25. Groups of species with large differences in species composition are separated first. Species marked with r were isolated from roots. The nematodes are in capitals.

zone of *A. arenaria* by Brown [1958], Dennis [1983] and Moreau and Moreau [1941] and from the root zone of *A. breviligulata* by Wohlrab et al. [1963]. These studies only dealt with isolations of fungi from soil. Brown [1958] isolated *Fusarium culmorum* more frequently from sand

Table 4. Groups of soil organisms that were significantly related to environmental variables when analysed with canonical correspondence analysis (CCA) (Fig. 1). The root zone of *A. arenaria* stands on lime-poor or calcareous foredunes was sampled. On calcareous foredunes, samples were taken from recently established stands and existing stands of more than 10 years old. The nematode names are in capitals

Calcareous foredunes Recently established	Calcareous foredunes Existing vegetation	Lime-poor foredunes Existing vegetation
Acremonium furcatum	*Pleospora* sp.	*Nectria inventa*
A.rutilum	*Fusarium chlamydosporium*	*Acremonium murorum*
A.strictum	*Aspergillus sydowii*	*Arthirinium phaeospermum*
Harzia acremonioides	*Chrysosporium pannorum*	*Verticillium lecanii*
Humicola grisea	*Trichothecium roseum*	*Chaetomium globosum*
Scopulariopsis sp.	*Phoma* spp.	*HELICOTYLENCHUS* sp.
Alternaria alternata	*Microdochium bolleyi*	*ROTYLENCHUS GOODEYI*

collected from the alkaline than from acid dunes. In this study, *Microdochium bolleyi* was usually isolated from roots, which is where it is also usually found for other plant species [Murray and Gadd, 1981]. Bussau [1990], Yeates [1968] and Zoon et al. [1993] isolated nematodes from dunes in Germany, Denmark, New Zealand and the Netherlands. Unlike Van der Putten et al. [1989], they did not detect *Meloidogyne* and *Heterodera* in the rhizosphere of *A. arenaria*. There seem to be two species of *Heterodera* present in the rhizosphere of *A. arenaria*: *H. avenae* and a *Heterodera* from the trifolii-group [H. Brinkman, pers. comm.], however, further taxonomic studies are required to confirm these observations. *Meloidogyne maritima* was found to be parasitising roots of *A. arenaria* in the dunes of Wales [Jepson, 1987]. Some of the nematodes species occurring in the root zone of *A. breviligulata* [Seliskar and Huettel, 1993] are different from those occurring in the root zone at of *A. arenaria*.

In both CCA and TWINSPAN-analyses no relation between groups of soil organisms and the vigour of the plants was found. There are two possible explanations. First, the between-location variation in the general survey was so large that it would require more locations (or better more locations within a few separate soil and vegetation types) to detect vigour effects. As repeated samples within a site gave similar results (Table 6), it is concluded that sampling is not the major source of variation. Secondly, in vigorous stands potentially harmful soil organisms were already present, as was shown by Van der Putten et al. [1989]. In that case no differences between communities of soil organisms from vigorous and early declining stands of *A. arenaria* could be expected. According to Van der Putten et al. [1989], roots of vigorous stands, which received 10 to 30 cm of windblown sand annually, are supposed to be colonized by soil organisms after they migrated towards the newly formed root layer. Since throughout the growing season only a slight shift in the composition of the community of soil organisms was found, soil organisms may already have migrated towards the newly formed root layer as early as March. It is concluded that both vigorous and early declining stands of *A. arenaria* contained similar communities of soil organisms.

The rhizosphere communities of *A. arenaria* from calcareous versus lime-poor stands and from dredged sea sand versus established stands foredunes were significantly different according to CCA. The differences were more significant for fungi than for nematodes. All nematode species, except *Rotylenchus goodeyi* and *Helicotylenchus* sp. from soil, were clustered in the centre of the axis together with only 6 species of fungi that have been classified as plant-pathogenic [Domsch et al., 1980]. In TWINSPAN, most of the soil organisms which occurred independent of environmental variables, were grouped in cluster 1 (Table 5).

The 'pathogenicity' of the soil collected from each location has also been tested in pot-experiments [Van der Goes and Van der Putten, 1992]. The growth reduction of *A. arenaria* seedlings in non-sterilized sand from vigorous stands was similar to that in sand obtained from early declining stands. Besides this, growth reduction of *A. arenaria* seedlings was observed in soil from each location [Van der Goes and Van der Putten, 1992]. It was therefore concluded that at all loca-

Table 5. TWINSPAN-analysis of soil fungi and nematodes that occurred in the root zone of *A. arenaria* independently of the environmental data. The numbers represent the abundance classes made in TWINSPAN (see materials and methods for further explanation). The samples are presented by numbers (1 to 16; see Table 1). The even numbers are samples taken from early declining stands. The isolations from roots are marked with r. The abbreviations consist of the first 3 characters of genus-name and 3 of species-name. The nematodes are in capitals. Blank columns and rows represent divisions made by TWINSPAN when Eigenvalue > 0.25. – = not isolated

cluster		samples	7	11	2	3	4	6	8	1	5	9	10	15	12	13	14	16
		PARA	5	–	5	5	5	5	5	4	4	–	–	–	–	5	–	–
	r	*PARA*	5	–	4	4	4	–	5	2	3	–	–	–	–	5	–	–
		Har arc	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–
1		*Sta cha*	–	–	–	–	–	5	5	–	–	–	–	–	–	–	–	–
		TEL VEN	5	–	5	5	5	5	5	5	5	–	–	–	–	–	–	–
	r	*Acr fur*	–	–	–	–	–	–	5	5	–	–	–	–	–	–	–	–
	r	*Cha fim*	–	–	–	–	–	–	–	–	5	–	–	–	–	–	–	–
	r	*Cla sp.*	5	5	–	–	–	–	–	5	5	–	–	–	–	–	–	–
	r	*Mucor*	2	–	2	5	5	4	–	–	2	–	–	–	–	–	–	–
	r	*ROT GOO*	3	–	–	3	–	–	5	–	–	–	–	–	–	–	–	–
		Mucor	5	5	–	–	2	1	–	–	–	1	–	1	–	–	1	–
2		*MEL* lv	5	5	4	4	4	3	4	2	1	–	3	4	–	–	–	3
	r	*PRAT*	1	1	2	1	–	1	1	–	1	5	2	4	–	–	–	1
	r	*HET* lv	5	1	–	4	1	–	1	1	–	–	4	1	–	–	1	–
		PRAT	–	–	3	4	4	3	–	3	2	5	3	5	–	–	–	–
3		*HET* m	–	5	3	–	–	–	–	–	–	–	5	5	–	–	–	–
	r	*Ulo sp.*	–	–	–	–	–	–	5	4	–	–	5	5	–	–	–	–
	r	*HELICO*	–	–	–	–	–	–	4	–	–	5	–	5	–	–	–	–
	r	*MEL* lv	5	1	–	1	1	–	1	1	–	2	2	1	–	–	–	–
	r	*HET* m	5	–	–	4	–	–	–	4	–	5	–	–	–	–	–	–
		HET lv	5	–	1	4	5	2	–	4	1	–	–	4	4	–	–	–
		APH	–	–	5	5	–	–	5	4	3	–	–	5	–	–	–	5
	r	*Mic bol*	–	–	5	–	4	4	–	3	5	–	4	3	–	5	–	4
	r	*FIL*	5	–	4	4	4	4	4	3	3	5	5	4	–	5	4	4
4	r	*TEL VEN*	5	3	4	5	5	5	5	4	5	5	5	–	5	–	–	4
		Mort	5	5	2	–	1	1	–	–	–	–	–	3	–	–	–	3
	r	*Fus cul*	5	–	–	–	5	–	–	–	–	–	4	–	–	4	–	–
	r	*Tri har*	5	–	2	5	2	–	–	3	3	–	3	–	5	–	–	5
	r	*APH*	3	–	–	4	3	4	–	–	–	–	–	–	–	–	–	5
5		*Fus cul*	–	–	–	2	2	5	5	–	2	–	–	3	5	5	4	–
	r	*Pen spp.*	2	4	4	–	4	2	5	5	5	–	–	3	5	5	5	5
		Abs cor	–	–	–	–	–	–	–	–	–	–	–	–	5	–	3	–
6		*Fus niv*	–	–	–	–	–	–	–	–	–	–	–	5	5	4	–	–
		Ulo	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	5
		Api mon	–	–	–	3	–	–	–	1	–	–	4	–	–	–	4	5
7		*Pen spp.*	1	1	–	1	1	1	1	1	–	1	2	3	5	5	5	4
		Tri har	–	–	–	1	–	–	–	1	3	–	–	3	5	1	5	–
8		*Pyreno*	–	–	–	–	–	–	–	2	2	–	4	5	–	–	–	5
	r	*Api mon*	–	–	–	–	–	–	–	–	–	5	–	–	–	–	–	3
		FIL	3	5	4	3	4	4	3	3	3	4	3	5	5	5	5	5
9		SAPRO	4	5	4	4	5	4	4	3	4	5	4	5	4	5	5	5
	r	SAPRO	4	3	2	4	4	4	3	2	3	5	5	5	5	5	4	5

tions degeneration could be related to soil-borne organisms. It may be that at all locations the same pathosystem is responsible for the degeneration. In that case, the potentially harmful soil organisms to *A. arenaria* will be found in the ubiquitous group. It may also be that differences in the com-

Table 6. TWINSPAN-analysis of soil-organisms collected from Voorne in May 1990. The vegetation of the different locations of Voorne originates from 1987 and has been established from seeds (location 3) or culms and rhizomes (location 5). Location 4 was an existing vegetation of more than 10 years old. The replicates were analysed separately with TWINSPAN. The numbers represent the classes made in TWINSPAN (see Materials and methods). The isolations from roots are marked with r. The nematode-genera are in capitals. Blank columns and rows represent divisions made by TWINSPAN with an Eigenvalue > 0.25. – == not isolated

location	3	5	5	5	5	3	3	3	4	4	4	4
replicate number	2	1	2	3	4	3	1	4	1	2	3	4
r *Acremonium furcatum*	3	–	–	–	–	5	5	5	–	–	–	–
A. furcatum	–	–	–	–	–	–	5	5	–	–	–	–
Aspergillus sydowii	–	–	–	–	–	2	5	–	–	–	–	–
Pyrenochaeta sp.	4	–	–	–	–	5	–	5	–	–	–	–
Stachybotrys chartarum	–	–	–	–	5	5	–	–	–	–	–	–
r *Cladosporium cladosporioides*	–	5	–	–	–	–	–	–	–	–	–	–
r *Stachybotrys chartarum*	5	–	–	–	–	–	–	–	–	–	–	–
r *Thielavia heterothallica*	–	–	–	–	5	–	–	–	–	–	–	–
Acremonium fusidioides	–	–	–	–	5	–	–	–	–	–	–	–
A. strictum	–	–	–	–	5	–	–	–	–	–	–	–
Chaetomium funicola	–	–	–	–	5	–	–	–	–	–	–	–
Fusarium culmorum	5	–	–	–	5	–	–	–	–	–	–	–
Phoma spp.	–	–	–	–	5	–	–	–	–	–	–	–
Trichoderma harzianum	5	–	–	–	–	–	–	–	–	–	–	–
Ulocladium sp.	3	5	5	–	–	–	–	–	–	–	–	–
PRATYLENCHUS sp.	–	–	5	–	–	–	–	–	–	–	–	–
TYLENCHORHYNCHUS MICROPHASMIS	–	–	–	–	5	–	–	–	–	–	–	–
FILENCHUS sp.	–	5	–	5	5	5	5	–	–	–	5	–
HETERODERIDAE males	–	–	5	–	–	–	–	–	–	–	–	–
TELOTYLENCHUS VENTRALIS	3	3	5	5	4	5	–	–	–	–	–	–
HETERODERA larvae	4	5	5	4	5	–	–	–	–	–	–	4
r *Microdochium bolleyi*	4	5	4	4	5	4	4	4	4	–	–	5
M. bolleyi	–	–	4	–	5	5	–	–	–	–	5	–
SAPROBIOTIC NEMATODES	5	4	4	4	4	5	5	5	3	3	3	4
r *Trichoderma harzianum*	–	–	–	5	–	–	5	–	–	–	–	5
Mucor hiemalis	5	–	–	–	–	–	5	–	5	–	–	–
r *Ulocladium* sp.	–	–	5	4	–	–	–	–	–	4	–	–
Acremonium murorum	–	–	4	–	–	–	–	–	–	–	5	–
Cladosporium cladosporioides	5	–	–	–	3	–	–	–	–	–	5	–
Thielavia heterothallica	5	–	–	–	–	–	–	–	5	–	5	–
HEMICRICONEMOIDES sp.	–	5	–	–	–	–	–	–	–	–	–	5
Fusarium dimerum	–	–	–	–	–	–	5	–	2	2	2	–
r *Fusarium culmorum*	–	–	–	–	–	–	–	–	5	–	–	–
r *Penicillium* spp.	–	–	–	–	–	–	–	–	5	5	5	4
Alternaria tenuissima	–	–	–	–	–	–	–	–	–	–	–	5
Penicillium spp.	–	–	2	–	–	1	–	–	5	5	5	5
ROTYLENCHUS GOODEYI	–	–	–	–	–	–	–	–	5	–	5	5
HELICOTYLENCHUS sp.	–	–	–	–	–	–	–	–	–	5	–	–
MELOIDOGYNE MARITIMA	–	–	–	–	–	–	–	–	5	–	–	–
APHELENCHUS sp.	–	–	–	–	–	–	–	–	–	–	5	–

munity of soil organisms between locations contribute to the degree of degeneration of *A. arenaria* on a local scale. Then all possible combinations of harmful soil organisms may be involved in the degeneration.

The additional analysis with TWINSPAN of the community of soil organisms which occurred indiscriminately in CCA and in cluster 1 by TWINSPAN, resulted in several clusters of concurring soil organisms (Table 5). If an omnipresent pathosystem is assumed as one of the possibilities discussed above, then 5 clusters of soil

organisms can be involved: i.e. clusters 2, 4, 5, 7 and 9 (Table 5). Both nematodes and fungi are supposed to be involved in the degeneration of *A. arenaria*, although the role of fungi only has not been clearly assessed yet [Van der Putten et al., 1990].

If fungi are not involved, then clusters 2, 4 and 9 are possible (Table 5), because the presence of plant parasitic nematodes within these clusters is sufficient. In cluster 2, three endoparasitic nematodes (*Meloidogyne maritime, Heterodera* spp. and *Pratylenchus* sp.) occur in combination with *Mucor hiemalis*. Endoparasitic nematodes are known to cause severe damage to many crops [Yeates, 1987], but hardly anything is known about their role in natural vegetations. Cluster 4 was formed by the nematodes *Heterodera* spp., *Filenchus* sp. and *Telotylenchus ventralis* with the potential plant-pathogenic fungi *Fusarium culmorum* and *Microdochium bolleyi* and the fungi *Mortierella* sp. and *Trichoderma harzianum*. Cluster 9 contained the root/fungal feeding *Filenchus* and the non-plant-feeding nematodes.

If fungi are involved, then cluster 2 (with *Mucor hiemalis*) and 4 are possible. As the fungus *M. hiemalis* (in cluster 2) is not known to infect plants [Domsch et al., 1980], only cluster 4 seems to be relevant. Combinations of nematodes and fungi as disease-complexes in agricultural crops, especially combinations of root-knot nematodes and *Fusarium* diseases, are well documented [e.g. Mai and Abawi, 1987; Powell et al., 1971]. Usually, saprophytic fungi do not contribute to degeneration. However, species which may enhance plant growth like *Trichoderma* [Windham et al., 1986] can, in combination with other soil organisms, inflict damage to crops [Powell et al., 1971]. Therefore, saprophytic fungi that were combined with pathogenic fungi or nematodes should be included in tests for pathogenicity as well.

Both CCA and TWINSPAN created lower dimensionality in a rather complex set of data and led to a higher interpretability. CCA arranged sites and/or species along environmental gradients. The most important limitation of CCA is that the environmental variables are assumed to be measured without error and to be constant within a site [Palmer, 1993]. In this study, the environmental variables are presented as zero's and one's and thus are constant with one site. The canonical analysis showed that these discrete environmental 'gradients' could be used to explain the complex set of data. Furthermore, the Monte Carlo permutation test does not depend on the type of distribution of the environmental gradients and, therefore, the significance of environmental variables to clusters of species are reliable.

TWINSPAN arranged sites and species into groups and demonstrated to be useful for creating subsets of soil organisms. Although each analysis made with TWINSPAN resulted in different combinations of soil organisms, and results of TWINSPAN are therefore hard to interpret, several combinations of pathogenic and parasitic soil organisms of possible interest could be derived from the combined application of CCA and a subsequent TWINSPAN-analysis. Thus, both CCA and TWINSPAN have shown to be valuable explanatory techniques and may be used to detect possible combinations of soil organisms which may be involved in the degeneration of *A. arenaria*. However, subsequent inoculation-experiments will be needed to elucidate the disease complex furtheron.

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References

Baye PR (1980) Comparative growth responses and population ecology of European and American beachgrasses (*Ammophila* spp.) in relation to sand accretion and salinity. PhD. thesis. University of Western Ontario, London, 309 pp

Bongers T (1988) De nematoden van Nederland. Stichting uitgeverij Koninklijke Nederlandse Natuurhistorische vereniging. Utrecht. 408 pp.

Brown JC (1958) Soil fungi of some British sand dunes in relation to soil type and succession. Journal of Ecology 46: 641–664

Bussau C (1990) Freilebende Nematoden aus Küstendünen und angrenzenden Biotopen der deutschen und dänischen Küsten. I. Gesamtüberblick und Chromadorida (Nematoda). Zoologische Anzeiger 225: 161–188

Dennis RWG (1983) Fungi of *Ammophila arenaria* in Europe. Revista de Biologia 12: 15–47

Domsch KH, Gams W and Anderson T-H (1980) Compendium of soil fungi. Vol. 1 & 2. Academic Press, London. 895 + 405 pp.

Eldred RA and Maun MA (1982) A multivariate approach to the problem of decline in vigour of *Ammophila*. Canadian Journal of Botany 60: 1371–1380

Gams W and Van Laar W (1982) The use of solacol (validamycin) as a growth retardant in the isolation of fungi. Netherlands Journal of Plant Pathology 88: 39–45

Hill MO (1979) TWINSPAN – a FORTRAN program for arranging multivariate data in an ordered two-way table by classification of individuals and attributes. Cornell University, Ithaca, New York. 90 pp

Huiskes AHL (1979) Biological flora of the British isles: *Ammophila arenaria* (L.) Link (*Psamma arenaria* (L.) Roem. et Shult.: *Calamagrostis arenaria* (L.) Roth). Journal of Ecology 67: 363–382

Jepson SB (1987) Identification of root-knot nematodes (*Meloidogyne* species). C.A.B. International, Wallingford, Oxon, 265 pp

Jongman, RHG, Ter Braak CJF and Van Tongeren OFR (1987) Data analysis in community and landscape ecology. Pudoc, Wageningen, 299 pp

Maas PWTH, Oremus PAI and Otten H (1983) Nematodes (*Longidorus* n. sp. and *Tylenchorhynchus microphasmis* Loof) in growth and nodulation of sea buckthorn (*Hippophaë rhamnoides* L.). Plant and Soil 73: 141–147

Mai WF and Abawi GS (1987) Interacting among root-knot nematodes and *Fusarium* wilt fungi on host plants. Annual Review of Phytopathology 25: 317–338

Maun MA and Baye P (1988) The ecology of *Ammophila breviligulata* Fern. on coastal dune systems. Critical Review in Aquatic Science 1: 661–681

Murray DIL and Gadd GM (1981) Preliminary studies on *Microdochium bolleyi* with special reference to colonization of barley. Transactions of the British Mycological Society 76: 397–403

Moreau M and Moreau F (1941) Première contribution à l'étude de la microflore des dunes. Revue de Mycologie 6: 49–94

Nelson PE, Toussoun TA and Marasas WFO (1983) *Fusarium* species. An illustrated manual for identification. Pennsylvania State University Press. University Park, London. 193 pp

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

Palmer MW (1993) Putting things in even better order: the advantages of canonical correspondence analysis. Ecology 74: 2215–2230

Powell NT, Melendez PL and Batten CK (1971) Disease complexes involving *Meloidogyne incognita* and certain soilborne fungi. Phytopathology 61: 1332–1337

Rozema J, Laan P, Ernst WHO and Appelo CAJ (1985) On the lime transition and decalcification in the coastal dunes of the province of North Holland and the island of Schiermonnikoog. Acta Botanica Neerlandica 34: 393–411

Seliskar DM and Huettel RN (1993) Nematode involvement in dieout of *Ammophila breviligulata* (Poaceae) on the mid-atlantic coastal dunes of the United States. Journal of Coastal Research 9: 97–103

's Jacob JJ and Van Bezooijen J (1984) Manual for practical work in nematology. Practicumhandleiding vakgroep nematologie, Landbouw Universiteit Wageningen. 77 pp

Ter Braak CJF (1988) CANOCO – a FORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal components analysis and redundancy analysis (version 2.1). Groep Landbouwwiskunde Wageningen. 95 pp

Van der Goes PCEM and Van der Putten WH (1992) Are nematodes involved in the degeneration of *Ammophila arenaria* (marram grass)? Mededelingen van de Faculteit van de Landbouwwetenschappen Universiteit van Gent, 57/3a: 847–856

Van der Putten WH (1990) Establishment and management of *Ammophila arenaria* (marram grass) on artificial coastal foredunes in the Netherlands. Proceedings Canadian Symposium on coastal sand dunes 1990: 367–387

Van der Putten WH, Van der Werf-Klein Breteler JT and Van Dijk C (1989) Colonization of the root zone of *Ammophila arenaria* by harmful soil organisms. Plant and Soil 120: 213–223

Van der Putten WH, Maas PWTh, Van Gulik WJM and Brinkman H (1990) Characterization of soil organisms involved in the degeneration of *Ammophila arenaria*. Soil Biology and Biochemistry 22(6): 845–852

Van der Putten WH and Troelstra SR (1990) Harmful soil organisms in coastal foredunes involved in degeneration of *Ammophila arenaria* and *Calammophila baltica* Canadian Journal of Botany 68: 1560–1568

Van der Putten WH, Van Dijk C and Peters BAM (1993) Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362: 53–55

Willis AJ (1989) Coastal sand dunes as biological system. Proceedings of the Royal Society of Edinburgh 96B: 17–36

Windham MT, Elad Y and Baker R (1986) A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76: 518–521

Wohlrab G, Tuveson RW and Olmsted CE (1963) Fungal populations from early stages of succession in indiana dune sands. Ecology 44: 734–740

Yeates GW (1968) An analysis of annual variation of the nematode fauna in dune sand, at Himatangi Beach, New Zealand. Pedobiologia 8: 173–207

Yeates GW (1987) How plants affect nematodes. Advances in Ecological Research 17: 61–113

Zoon FC, Troelstra SR and Maas PWTh (1993) Ecology of the plant-feeding nematode fauna associated with sea buckthorn (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) in different stages of dune succession. Fundamental and Applied Nematology 16(3): 247–258.