NEMATODES AND DECOMPOSITION IN INTERTIDAL ECOSYSTEMS

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NEMATODES AND DECOMPOSITION IN INTERTIDAL ECOSYSTEMS

Proefschrift

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in de landbouw- en milieuwetenschappen
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STELLINGEN

1 Bioturbatie of "het omwerken" is het belangrijkste mechanisme waarmee nematoden de bacteriële activiteit en daarmee ook de afbraak van organisch materiaal kunnen stimuleren.

Dit proefschrift

2 De totale nematodenpopulatie die aanwezig is op schorren wordt ernstig onderschat als alleen in het sediment naar nematoden wordt gezocht.

Dit Proefschrift

3 Als de verhouding tussen bacterieëtende nematoden en schimmeletende nematoden de verhouding weergeeft van bacteriële afbraak van *Spartina anglica* en afbraak door schimmels dan wordt, in tegenstelling tot wat algemeen wordt aangenomen, *S. anglica* vooral door bacteriën afgebroken.

Newell S.Y., R.D. Fallon & J.D. Miller, 1989. Mar. Biol. 101: 471-481

4 Nu Nederland lid is van het Scientific Committee on Antarctic Research (SCAR) zouden de onderzoeksinspanningen van Nederlandse onderzoeksinstituten in Antarctica een structureel karakter moeten krijgen.

5 Het is beter om 'correspondence analysis' te gebruiken, en de tekortkomingen van deze multivariate analyse methode voor lief te nemen, dan gebruik te maken van 'detrended correspondence analysis', omdat er geen ondubbelzinnige procedure bestaat waarop 'detrending' kan worden uitgevoerd.

Jackson, D.A. & K.M. Somers, 1991. Am. Nat. 137: 704-712

6 Het is opvallend dat er bij het bemonsteren van mariene nematoden nauwelijks aandacht wordt besteed aan de grote variabiliteit op korte afstand, terwijl dit bij het bemonsteren van terrestrische nematoden gemeengoed is.

Huys R., P.J.M. Herman, C.H.R. Heip, & K. Soetaert, 1992.
ICES J. mar. Sci. 49: 23-44;
Southey J.F., 1986. Reference book 402, Crown, London

7 Kraamverzorg(st)ers worden ondergewaardeerd in hun functie als onderwijsgevenden aan Ouders in Opleiding (OIO's)

8 De fixatie van veel vegetatiekundigen op het indelen van vegetaties in zogenaamde plantengemeenschappen heeft geleid tot verminderde aandacht voor de reactie van individuele plantensoorten op milieuveranderingen.

Schaminée J.H.J., 1993. proefschrift, Katholieke Universiteit Nijmegen

9 Experimenten in de ecologie kunnen vaak met minder inspanning evenveel of meer opleveren indien vooraf de methoden voor de statistische analyse worden vastgelegd.

10 Het invoeren van 'Good Laboratory Practice' (GLP) leidt in ieder geval tot een uitbreiding van de papier consumptie van een instituut.

Stellingen behorend bij het proefschrift van Rob Alkemade: *Nematodes and decomposition in intertidal ecosystems.*
Wageningen, 14 september 1993

VOORWOORD

Dit proefschrift is het resultaat van bijna vier jaar onderzoek naar de rol van nematoden in decompositie processen aan het Centrum voor Mariene en Estuariene Oecologie van het Nederlands Instituut voor Oecologisch Onderzoek. Het onderzoek wat op King George Island, Antarctica heeft plaatsgevonden en onderdeel uitmaakt van dit proefschrift is uitgevoerd tijdens de Nederlandse Antarctica expeditie van 1990-1991. Deze expeditie werd georganiseerd door de Stichting Onderzoek der Zee en gefinancierd door het Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieuhygiëne.

Natuurlijk kon dit onderzoek nooit uitgevoerd en voltooid worden zonder de inspanning van vele anderen. Zonder iemand binnen en buiten het CEMO te kort te willen doen wil ik enkelen met name noemen.

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Alle leden van de werkgroep Litorale Vegetaties van het CEMO wil ik bedan-

ken voor de plezierige sfeer waarin gewerkt kon worden. De collega's van het CEMO zorgden ervoor dat ik iedere ochtend met plezier de reis van Rotterdam naar Yerseke aanvaardde.
Mijn promotor Prof. Dr. Ir. A.F. van der Wal volgde het onderzoek met grote belangstelling. Ton, ik wil je bedanken voor de prettige en ontspannen manier waarop de teksten besproken werden. De discussies met jou en Marten leidde altijd tot meer samenhang tussen de verschillende onderdelen.
Tenslotte wil ik Katrien bedanken. Ik denk dat dit proefschrift er nog lang niet gelegen had, wanneer jij niet ook met een proefschrift bezig was. Omdat jij ook in het weekend veel tijd besteedde aan je onderzoek kon ik niet achterblijven.

Rob Alkemade
22 juni 1993
Rotterdam

CONTENTS

GENERAL INTRODUCTION

Temperate salt marshes belong to the most productive natural vegetations in the world (Odum 1971; Long and Mason, 1983). An annual above-ground production of more than 1 kg dry weight per m^2 per year is not exceptional (De Leeuw & Buth, 1991). Only a few percent of the annual production is consumed by herbivores, the major proportion of the above-ground plant parts dies after senescence. By far the greatest part of the resulting plant litter decomposes at the production site. Export of plant detrital matter from coastal and estuarine salt marshes of the Netherlands probably is insignificant (Hemminga et al., 1992, 1993).

Decomposition of dead plant material is primarily a microbial process, in which fungi and bacteria, as heterotrophic organisms, play a dominant role. The rate of decomposition depends mainly on the chemical composition of the detritus (Valiela et al., 1985; Hemminga and Buth, 1991), but also on many other factors, such as temperature and humidity (Christian, 1984; Newell et al., 1985). The micro- and meiofauna, feeding on the microbial decomposers may, indirectly, influence the decomposition process as well.

In marine sediments, nematodes are by far the most abundant group of metazoans, especially when large amounts of organic matter are available. Nematodes are found in high numbers on decomposing material of salt marsh plants (e.g. Buth & de Wolf, 1985; Montagna & Ruber, 1980). However, in spite of their abundance, little is known about the ecological role of these nematodes. The study of free living nematodes in the marine environment was until recently primarily dedicated to taxonomic aspects, but a large progress with regard to the knowledge of the functioning of nematodes in marine ecosystems was achieved during the last two decades (Heip et al., 1992). A possible stimulatory role of nematodes in regeneration of nutrients and decomposition was already suggested by Johannes (1965). In the late seventies, early eighties several authors emphasized the importance of nematodes in marine benthic ecosystems and suggested several working mechanisms on which nematodes may be able to stimulate decomposition processes (Gerlach, 1978; Riemann and Schrage, 1978; Tietjen, 1980; Lee 1980). Experimental evidence of a possible stimulatory effect by the decomposition rate of macrophyte derived detritus by microbivorous nematodes was first reported by Findlay & Tenore (1982).

The present study is focused on aspects of the ecology and the population dynamics of microbivorous nematodes present on decomposing plant material and the role of these nematodes in decomposition processes. The major part of this study is dedicated to nematodes associated with decomposing, above-ground parts of *Spartina anglica*. *S. anglica* is found in the lower zones in Western European salt marshes. This vegetation is flooded frequently, so the tide is an important factor for the decomposition of *Spartina* and the ecology of its associated nematodes.

Not all nematodes found on decaying *Spartina* litter will have an influence on the decomposition process. Many species presumably are present because the litter offers a living space. In chapter I a study is presented on the identification of the nematodes which are probably of importance to the decomposition process.

One of the nematodes identified in the first chapter is *Diplolaimelloides bruciei*. This nematode was found abundantly on all plant parts of the standing plants of *S. anglica*. Since these plants often are partially submerged by flood tides, the population is continually at risk of washing away. The population dynamics of *D. bruciei* on standing dead *Spartina* plant parts are studied in chapter II.

In chapter III, a study is reported on a similar association between nematodes and macrophyte derived detritus in an entirely different ecosystem. Large amounts of seaweed wrack are washed ashore on Antarctic beaches. The nematode population densities and the decomposition rate of the seaweed detritus were related to seaweed- and location characteristics, such as the carbon to nitrogen ratio, the salinity and the presence of melt water streams.

The effect of bacterivorous nematodes on the decomposition of *Spartina* detritus was investigated in a series of laboratory experiments reported in the chapters IV and V. The effect of *Diplolaimelloides bruciei* on the decomposition of leaves was investigated by measuring weight losses as well as the production of CO_2 in the presence and absence of the nematodes. The decomposition of organic matter in the upper layer of the sediment was studied in the presence of *Diplolaimella dievengatensis*, a sediment dwelling nematode. The abundance of this species was also found to be positively correlated with decomposition rates, as is shown in chapter I. Bioturbation effects of these nematodes were determined by measurements of both O_2 uptake and O_2 micro-gradients into the sedi-

ment. Simultaneous measurements of CO_2 production, O_2 consumption and the O_2 micro-gradients were used to estimate the contribution of bioturbation to the mineralization of organic substrate in the sediment (chapter V).

Microbivorous nematodes stimulate decomposition under certain circumstances, but not always, as was shown by data in our study and those of the literature. A simulation model was constructed and used to investigate the possible mechanisms by which nematodes affect the decomposition process (chapter VI). The validity of the model was verified by the use of laboratory and field data on the decomposition of *S. anglica.*

CHAPTER ONE

The correlation between nematode abundance and decomposition rate of *Spartina anglica* leaves

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ABSTRACT

Dead *Spartina anglica* leaves which are not washed away by the tides, decompose in the canopy as standing dead crop, or decompose on the sediment-surface. In the sediment many nematode species are present, which may colonize the *Spartina* litter. Some of these species may affect the decomposition of *Spartina* detritus. To identify these nematode species a field experiment was carried out. Small mesh containers filled with green or aged *Spartina* leaves to obtain different decomposition rates. The containers were placed in the Stroodorpepolder salt marsh in the Oosterschelde, a tidal inlet of the Southern North Sea. After 3 months the containers were collected and nematodes were counted and identified. The experiment was repeated in four subsequent seasons.

Fifty-nine nematode species were found in the containers; most of the species were found in all treatments. Total numbers of nematodes did not differ among treatments. Using multivariate analysis (RDA), however, we found differences of nematode community structure between the treatments. Bacterivorous nematodes, particularly monhysterid species, prevailed in the containers with the highest decomposition rates. A positive correlation was found between decomposition rate and the numbers of *Diplolaimella dievengatensis* in spring, summer and autumn. The numbers of *Diplolaimelloides bruciei, Monhystera parva, Desmolaimus zeelandicus* and *Theristus acer* showed positive correlations with decomposition rates in one or two of these seasons. In winter no significant correlations were found. Thus, of the 59 nematode species present in the litter the abundances of only 5 species seemed to be affected by decomposition rate of the litter. It is suggested that these nematode species primarily react to the increased microbial activity coinciding with higher decomposition rates. In addition, these species may further enhance the microbial decomposer activity.

INTRODUCTION

Spartina anglica is a common halophyte of the lower zones of Western European salt marshes. Dead above-ground material of *S. anglica*, which is not

removed by the tides, either decomposes in the canopy, or on the sediment surface. Recent studies on the decomposition of *Spartina* material on the sediment surface showed that litter-associated nematodes are always found in high numbers (Montagna & Ruber, 1980; Reice & Stiven, 1983; Buth & de Wolf, 1985; Hemminga & Buth, 1991). A correlation between total nematode densities and decomposition rates, however, could not be established (Reice & Stiven, 1983; Buth & de Wolf, 1985). In these studies nematodes were considered as one taxon, which masks possible differences between individual species with respect to their dependence of, or their role in litter decomposition. The number of specimens of those species, that feed on microbial decomposers, presumably is determined by the decomposition rate, as at higher decomposition rates the microbial production probably will be higher, thus providing more food to microbial grazers.

In the present study, experiments were carried out to investigate changes in the nematode community in relation to changing decomposition rates of *S. anglica* leaf litter. An attempt was made to detect those nematode species, whose numbers correlated with decomposition rate. Differences in decomposition rates were obtained by using leaf litter of different ages. Decomposition rate decreases during decomposition: the readily available substances, such as proteins, sugars, etc. are lost first from the litter, leaving the more refractory components, which decompose at much lower rates. Nematode densities are subject to conspicuous seasonal fluctuations (Buth & de Wolf,1985; Vincx, 1989; Heip et al., 1985). Some species are found only in certain seasons. The experiments, therefore, were repeated in all four seasons.

Redundancy analysis (RDA) was used to evaluate the relation between nematode community structure and decomposition rates.

MATERIAL AND METHODS

Leaf material

Green leaves of *Spartina anglica* were collected in the Stroodorpepolder salt marsh in January 1988. This marsh is situated in the Oosterschelde, a tidal inlet of the Southern North Sea. Immediately after harvesting, the leaves were washed and cut into fragments of 2 cm. To obtain leaf litter of progressive decomposition stages, the material was split into three portions. One part was immediately dried at 50 °C (48 h) and stored. The other portions were aged for 2.5 months and 5 months, respectively, by putting the leaf fragments into a bag of nylon gauze (2 mm mesh) and placing them, in the laboratory, in a tank on sediment originating from the Stroodorpepolder salt marsh. The sediment was flooded each day (3 h) with water from the Oosterschelde. The ambient temperature ranged from 15 to 25 °C; the temperature of the flooding water ranged from 15 to 20 °C. After 2.5 and 5 months part of the leaf-material was removed and dried at 50°C for 48 h. A sample of leaf material was used for determining the initial contents of carbon-, nitrogen and phosphate.

Experimental design

Mesh containers were filled with 5 g dry weight (DW) of one of the three types of plant material. In addition, inert fragmented plastic drinking straws were used in control treatments. The containers used were permeable, consisting of 2 cm segments of perspex cylinders with a diameter of 7 cm, closed at both ends with 0.8 mm mesh gauze.

Four subsequent experiments were conducted at the same site in the Stroodorpepolder salt marsh. The site (10 m^2) was situated near the edge of the marsh, where *S. anglica* forms a monospecific stand. In each experiment the mesh containers were laid out in 5 randomized blocks, each block being a row parallel to the marsh edge. Within the rows one container of each treatment was placed in random order on the sediment surface. The distance between the rows and between the containers within a row was 0.5 m. The containers were anchored by two wire wickets to the marsh sediment to prevent them from being

washed away at high tide. The experiments started on March 15, June 20, September 19 and December 20 (1988). At these dates sediment samples were also collected to compare the number of nematodes in the upper sediment layer with the number found in the containers. The field exposure time of the containers was three months. Containers with 5 months aged leaves were included only in the summer, autumn and winter experiments.

Decomposition rate and chemical analyses

After retrieval of the mesh containers a small sample of the detritus was washed with tap water and dried for chemical analysis. The remaining part of the detritus was fixed with warm formalin at a final concentration of 4-5 %. After at least 7 days the detritus was rinsed with tap-water over a household sieve (mesh 1.5 - 2 mm) to separate the leaf material from the sediment and nematodes. The leaf material was retained on the sieve, whereas nematodes and sediment passed through. The leaf material was dried and weighed.
Decomposition rates were calculated as the loss of dry weight of leaf material from the mesh containers and expressed as g weight loss per 100 days, using a negative exponential relation for extrapolation (cf. Swift et al., 1979). Carbon and Nitrogen content of the samples were analyzed with a Carlo Erba CN-analyzer, type 1500 A. Phosphate content was determined colorimetrically.

Nematodes

The nematodes were extracted using an Oostenbrink elutriator (Fricke, 1979; 's Jacob & van Bezooijen, 1986). The nematodes were recovered by passing the suspension with nematodes (see above) through a series of four 45 µm mesh sieves. The debris retained on the sieves was collected in 250 ml centrifugation tubes with tap water. After centrifugation (5 min. at 3000 rpm) the supernatant was discarded and the nematodes in the pellet were extracted by two repeated centrifugation steps (1 min. at 3000 rpm) with a $MgSO_4$ solution (specific gravity of 1.28).
Nematodes were counted and 250 individuals were identified to species level using an inverted microscope.

Statistical analysis

Seasonal and treatment differences of losses of weight, nitrogen, carbon and phosphate and the total numbers of nematodes were evaluated using analysis of variance. Redundancy analysis (RDA) was used to evaluate the relation between the nematode community structure and the decomposition rate of the leaf material. RDA is the canonical form of principal component analysis (Jongman et al., 1987). Linearity between environmental gradients and species abundances is assumed for RDA. Since the experiments were carried out on a limited surface area, the environmental gradients were expected to be small. If gradients are small, the assumption for linearity is not very stringent. (ter Braak & Prentice, 1988; van der Meer, 1990). A log(X+1) transformation was used in order to stabilize variances. The log-transformation implies that the assumption of linearity between environmental variables and species abundances must hold for the linearity between the variables and log-numbers. Decomposition rate was included as the only independent variable. Corrections for seasonal effects and effects which may arise from the row-position of the mesh container in the field were made by taking them as covariables in the computer program CANOCO (ter Braak, 1990). The result of the RDA then only describes the variation explained by decomposition rate and the variation due to error terms. A Monte Carlo permutation test was carried out to test the significance of decomposition rate as the explaining variable. The species found to be correlated with decomposition rate were analyzed in more detail by regression of the numbers per individual species on the decomposition rate.
ANOVA and regression analyses were carried out with SYSTAT statistical package (Wilkinson, 1990). RDA and the Monte Carlo permutation test were carried out with CANOCO 3.10 (ter Braak, 1990)

RESULTS

After a few weeks of exposure in the field the leaf fragments were covered with a layer of sediment in most of the containers. Since the *Spartina* vegetation was

flooded frequently during the course of the experiments, no desiccation of the sediment surface took place.

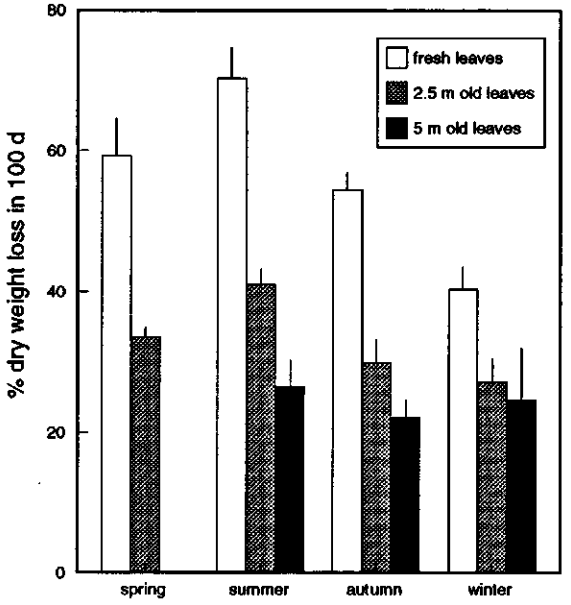

Figure 1: Decomposition rates of *Spartina anglica* expressed as % losses of dry weights of litter after 100 days. Means and standard deviations (n=5).

Decomposition rates

The decomposition rates of the three detrital types in the successive seasons are shown in Figure 1. Statistically significant treatment and seasonal differences were observed ($P < 0.001$). In all seasons decomposition rates of fresh leaves were higher than those of the aged material (Tukey test; $P < 0.05$). The 2.5 months aged leaves decomposed slightly faster than the 5 months aged material, but only in summer was this difference significant (Tukey test; $P < 0.05$). The differences between the detrital types were most pronounced in the summer season. The decomposition rates of the fresh leaves showed the most conspicuous seasonal fluctuations, with the highest rates in summer and the lowest rates in winter. With respect to the fresh leaf material and to the 2.5 months aged material differences in decomposition rates between the various seasons were

nearly all significant (Tukey test; $P < 0.05$). Decomposition rates of the 5 months aged material were not significantly different between the seasons. No weight losses from the inert material were observed.

Carbon, nitrogen and phosphate losses yielded similar patterns, the losses of these nutrients were highly correlated with dry weight losses (Bonferroni $P < 0.001$; table 1). Carbon, nitrogen and phosphate losses were highest in summer and lowest in winter. The losses of these nutrients were highest from fresh leaf-material.

Table 1: Correlation coefficients between decomposition rates and nutrient losses; all coefficients are significant (Bonferroni $P < 0.001$).

	Dec. rate	Carbon	Nitrogen
Carbon	0.99		
Nitrogen	0.93	0.94	
Phosphate	0.92	0.94	0.93

Nematodes

The total number of nematodes found in the mesh containers showed clear seasonal fluctuations (Table 2). The highest numbers were found in summer, whereas the lowest numbers were present in autumn. The numbers found in the mesh containers were in the same range as the numbers found in the upper layer of the surrounding sediment. No statistical differences were found between the treatments. Fifty-nine nematode species were found in the mesh containers (table 3). Most species are known from intertidal estuaries of the southern North sea. (Vincx, 1986; Bouwman, 1983). The majority of these species are known to feed on a variety of food sources. Carnivorous and omnivorous species are represented by *Enoplus communis* and *Adoncholaimus fuscus*. Herbivores are represented by members of the family Chromadoridae. Bacterivorous species are represented by some members of the family Monhysteridae. Selective deposit feeders, with a very small buccal cavity are represented by the *Halalaimus* species and by *Oxystomina* sp.

Table 2: Total number of nematodes found in the mesh containers in the various treatments and seasons and the numbers found in surrounding sediment in the same volume as the mesh container content.

	Fresh	2.5 m old	6 m old	control	sediment
Spring	22700	12700		14200	19400
Summer	46900	34900	27800	41300	28700
Autumn	9900	9400	9200	9500	20300
Winter	21200	13100	15300	18200	11300

RDA analysis was carried out on the whole species assemblage. The relation between the log-number of the most dominant species and the decomposition rate showed a linear pattern, therefore we could use and interpret RDA without restriction. Figure 2 and 3 show the RDA ordination diagrams obtained for the mesh containers and for the species, respectively. The arrows in the figures indicate the direction of increasing decomposition rates. The first ordination axis, explained by the decomposition rate, accounted for 6.9 % of the total variance. The Monte Carlo permutation test showed that, in spite of this small percentage of explained variance, the nematode community changed significantly with changing decomposition rate (F ratio = 5.08, $P < 0.01$).

In fig. 2 the different treatments were ordered along the first axis in such a way that the control treatments appear on the left hand side and the fresh leaf treatments appear on the right hand side. In figure 3 the ordination diagram of the species is shown. The position of a species in the figure reflects the contribution of the species to the variance explained by the first two axes. The species occurring at the left hand side of figure 3 are negatively correlated with decomposition rate and species appearing at the right hand side are positively correlated with decomposition rate. So the number of specimens of the majority of the species is not correlated with decomposition rates, as they appear near the origin. The species *Leptolaimus mixtus*, *Sphaerolaimus sp.* and *Hypodontolaimus inaequalis* have high negative scores on the first axis; these species may avoid from places with high organic inputs and are found more abundantly in the control treatments.

Table 3: Species list of the nematodes found in the litter containers, with abbreviations as used in figure 3.

Species:	Abbreviation	Species	Abbreviation
Enoplus communis	*Enop comm*	*Metochromadora remanei*	*Meta rema*
Anoplostoma viviparum	*Anop vivi*	*Metochromadora vivipara*	*Meta vivi*
Anticoma acuminata	*Anti acum*	*Microlaimus globiceps*	*Micr glob*
Dolicholaimus marioni	*Doli mari*	*Microlaimus sp.*	*Micr sp*
Halalaimus gracilis	*Hala grac*	*Nudora bipapillata*	*Nudo bipa*
Halalaimus longicaudatus	*Hala long*	*Monoposthia costata*	*Mono cost*
Oxystomina elongata	*Oxys elon*	*Leptolaimus mixtus*	*Lept mixt*
Adoncholaimus fuscus	*Adon fusc*	*Leptolaimus sp.*	*Lept sp*
Viscosia viscosa	*Visc visc*	*Aegialoalaimus sp.*	*Aegi sp*
Calyptronema maxweberi	*Caly maxw*	*Quadricoma scanica*	*Quad scan*
Bathylaimus australis	*Bath aust*	*Diplolaimella dievengatensis*	*Dipl diev*
Tripyloides marinus	*Trip mari*	*Diplolaimelloides bruciei*	*Dipl bruc*
Atrochromadora microlaima	*Atro micr*	*Monhystera disjuncta*	*Monh disj*
Chromadora nudicapitata	*Chro nudi*	*Monhystera parva*	*Monh parv*
Chromadorina sp.	*Chro ina*	*Monhystera sp.*	*Monh sp*
Chromadorita sp.	*Chro ita*	*Daptonema sp. 1*	*Dapt sp1*
Dichromadora geophila	*Dich geop*	*Daptonema sp. 2*	*Dapt sp2*
Dichromadora scandula	*Dich scan*	*Daptonema sp. 3*	*Dapt sp3*
Hypodontolaimus inaequalis	*Hypo inae*	*Daptonema sp. 4*	*Dapt sp4*
Hypodontolaimus sp.	*Hypo sp*	*Daptonema sp. 5*	*Dapt sp5*
Neochromadora sp.	*Neoc sp*	*Theristus acer*	*Ther acer*
Neochromadora poecilosoma	*Neoc poec*	*Theristus pertenuis*	*Ther pert*
Prochromadorella paramucrodonta	*Proc para*	*Trichotheristus sp.*	*Tric sp*
Ptycholaimellus ponticus	*Ptyc pont*	*Sphaerolaimus sp.*	*Spha sp*
Spilophorella paradoxa	*Spil para*	*Desmolaimus zeelandicus*	*Desm zeel*
Paracantonchus caecus	*Para caec*	*Metalinhomoeus biformis*	*Meta bifo*
Praeacantonchus sp.	*Prae sp*	*Paralinhomoeus sp.*	*Para linh*
Sabatieria sp.	*Saba sp*	*Ascolaimus sp.*	*Asco sp*
Desmodora communis	*Desm comm*	*Axonolaimus typicus*	*Axon typi*
		Pellioditis marina	*Pell mari*

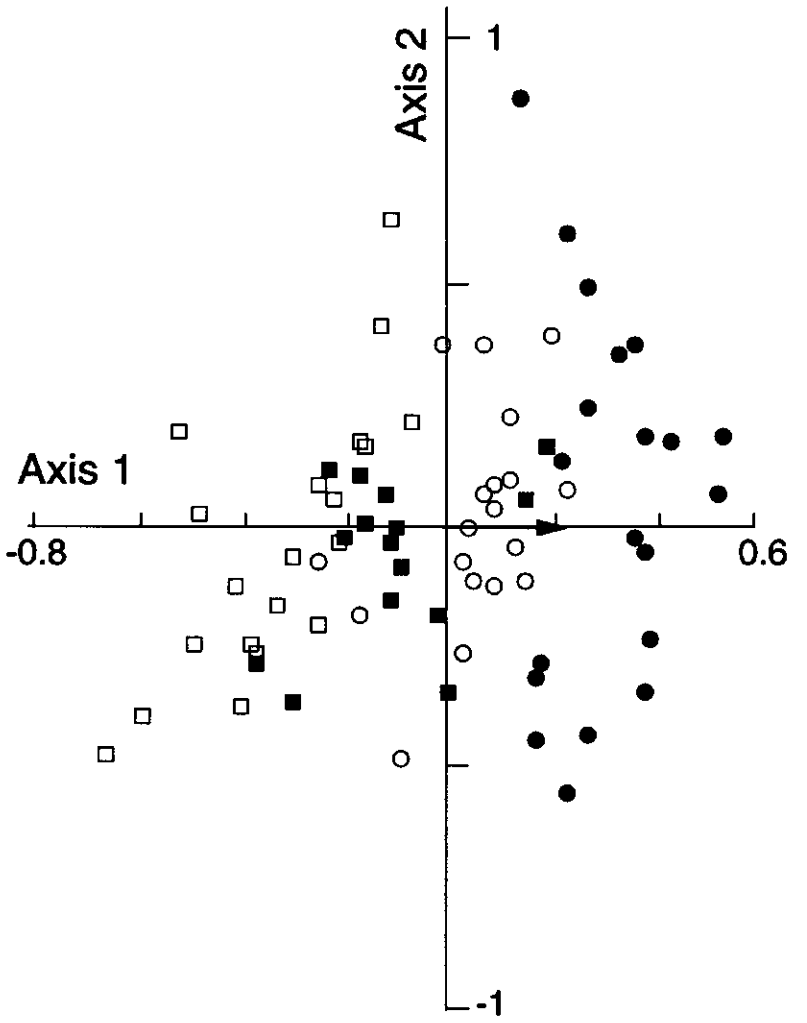

Figure 2: Ordination diagrams resulting from RDA on the nematode abundances in mesh containers, diagram of the mesh container scores (□ = controls, ● = fresh leaves, ○ = 2.5 m old leaves, ■ = 5 m old leaves). The arrowhead indicates to the direction of increasing decomposition rates.

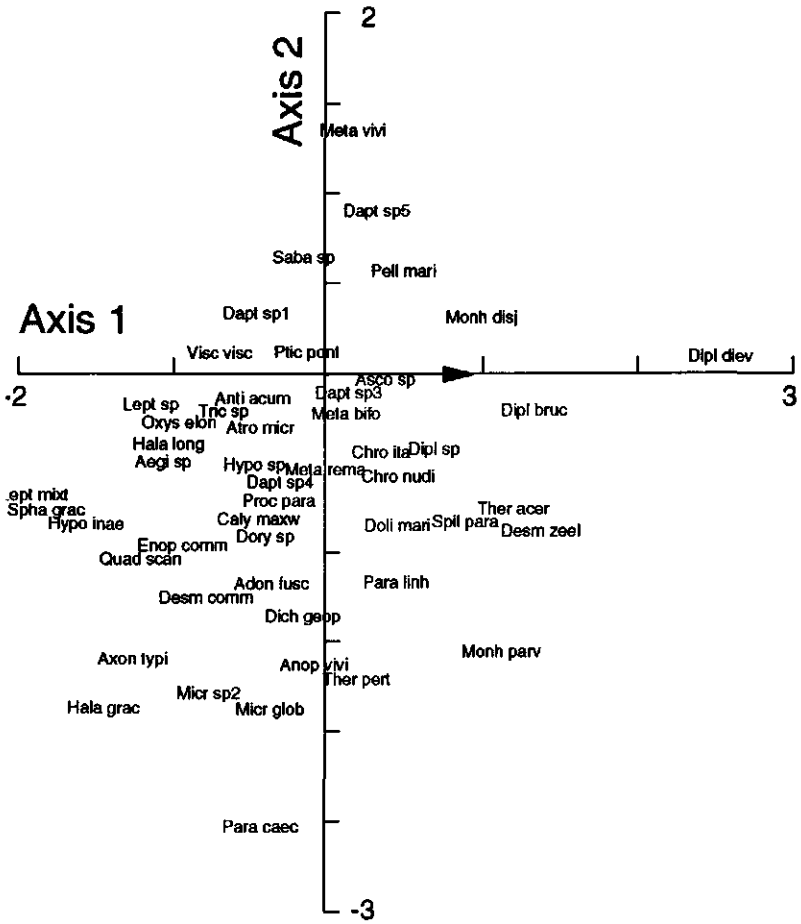

Figure 3: Ordination diagrams resulting from RDA on the nematode abundances in mesh containers, diagram of the species scores. An explanation of the abbreviations of species names is given in table 3. The arrowhead indicates to the direction of increasing decomposition rates.

The species *Diplolaimella dievengatensis* is separated from all other species and has the highest positive scores on the first axis. Other species with high positive scores on the first axis are, in decreasing order: *Desmolaimus zeelandicus*, *Diplolaimelloides bruciei*, *Theristus acer* and *Monhystera parva*. Thus, the numbers of only these species are positively correlated with decomposition rates. These five species are examined in more detail. Figure 4 shows the relation between the log-number of individuals of the five species and the weight loss of *Spartina* from the mesh containers. The regression lines were fitted for each separate season. All data, including zero observations, were used in these

analyses. The slopes of the regression lines are the increases of log-numbers of individuals per g weight loss of detritus.

The numbers of *D. dievengatensis* were most clearly related to decomposition rate (Figure 4A). The regression lines fit well for the experiments carried out in spring, summer and autumn ($P < 0.001$) but not in winter ($P = 0.15$). In spring, summer and autumn the slopes of the regression lines indicate that 4.6 to 9.6 times more specimens of *D. dievengatensis* occurred with an increased weight loss of 1 g in 100 days.

D. bruciei (Figure 4B) is less frequently found in the mesh containers than *D. dievengatensis*. Only in autumn were this species was found in more than 50 % of the containers. In this season the correlation between numbers and decomposition rate was high and the estimated slope of the regression line indicates that 7.9 times more specimens occurred with an increased weight loss of 1 g in 100 days (figure 4B). In summer *D. bruciei* was completely absent. In spring and winter the occurrence of *D. bruciei* was low, and the calculated regression coefficients were not significantly different from zero.

The numbers of *M. parva* (Figure 4C) found in the mesh containers ranged from 0 to more than 40,000 specimens per container. Only in treatments with fresh leaves did the numbers exceeded significantly the numbers found in control treatments. In some mesh containers more than 50 % of the total number of nematodes were individuals of *M. parva*. The high numbers of *M. parva* occurred in winter and spring and not in summer and autumn. The linear regression equations poorly describe the relation between the number of specimens and weight losses. Only in spring is the regression coefficient significantly different from zero. In each season, however, the highest numbers were found in the mesh containers with the highest weight losses i.e. the fresh leaf treatment.

Desmolaimus zeelandicus occurred in almost all samples. The regression coefficients are significantly different from zero in summer and autumn, but not in spring and winter (figure 4D). The slopes of the regression lines in summer and autumn were less steep than the slopes found for the species belonging to the Monhysterid species. From the calculated slopes it can be estimated that in summer and autumn 1.5 to 1.6 more specimens occurred with an increased weight loss of 1 g in 100 days.

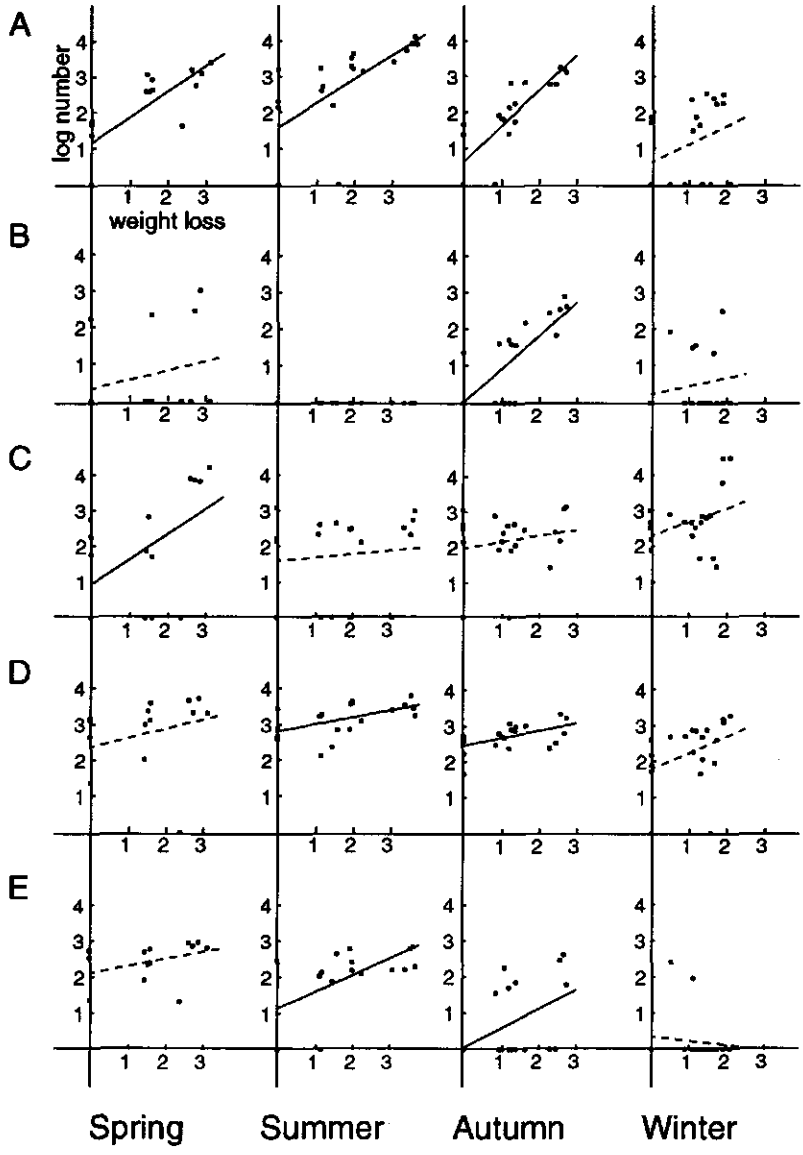

Figure 4: Plots of the log number of specimens against the decomposition rates. Horizontal axis denotes dry weight losses (g per mesh container), vertical axis denotes the log number of nematodes per mesh container. Lines are linear regression lines. Broken lines indicate non significant regression coefficients. A: *Diplolaimella dievengatensis*, B: *Diplolaimelloides bruciei*, C: *Monhystera parva*, D: *Desmolaimus zeelandicus*, E: *Theristus acer*.

Theristus acer occurred only in spring and in summer in more than 50 % of the containers. In the winter *T. acer* was almost absent. The regression coefficient, fitted for the summer data, is significantly different from zero, and is intermediate between the slopes found for the monhysterids and for *D. zeelandicus* (figure 4E). From the regression equation it can be derived that in summer 2.9 times more specimens occurred with an increased weight loss of 1 g in 100 days. In autumn the slope is also significantly different from zero and is very close to the figure found in summer. However, *T. acer* occurred in only 7 of the 20 mesh containers.

DISCUSSION

The decomposition rates found in this study ranged from 25 to 85 % weight losses after 100 days and are in the range found for *Spartina spp.* in the literature. Montagna & Ruber (1980) summarized decomposition rates from 6 studies on the decomposition of *S. alterniflora* which covered different seasons. The weight losses, after 100 days, in these studies ranged from 25 - 90 %, thus our data fall within this range. Hemminga & Buth (1991) reviewed studies on decomposition of salt marsh halophytes in the Netherlands. They calculated mean decomposition rates for *S. anglica* leaves ranging from 0.25 to 0.45 % d^{-1} over a period of 6 months. Assuming negative exponential decay, this would result in weight losses of 22 - 36 % after 100 days. Obviously the wide range in our study is a result of the separation into different quality classes and the influence of different seasons. In the studies reviewed by Hemminga and Buth (1991) samples of a mixture of quality types were used; other studies were performed in only one season.

A wide range of nematode species occurred in the mesh containers. The abundances of most of these species were not correlated with decomposition rate, they occurred in equal densities in treatments with decomposing material and in the control treatments. Apparently, the decomposing material forms only a living space for them. Our observations that the numbers of individuals of these species numerically dominate the total nematode fauna in the litter is consistent with the results of Buth & de Wolf (1985) and Montagna & Ruber

(1980), who found that the total number of nematodes in halophyte litter was dependent on the available space expressed as the amount of litter and not on the age of the decomposing detritus.

The RDA analysis showed that of the many species present in the litter, the number of specimens of only a few species were positively correlated with decomposition rate. These species were found in high numbers in the treatments with the highest decomposition rates, occurred in lower numbers in the treatments with aged leaf material and were practically absent in the control treatments. To date such a direct relation between decomposition rate and nematode numbers has not been found in field experiments. Three of the associated species, *D. dievengatensis*, *D. bruciei* and *M. parva*, are members of the family Monhysteridae. *D. zeelandicus* belongs to the family Linhomoeidae and *T. acer* is a member of the family Xyalidae. Members of the family Monhysteridae are considered non-selective deposit feeders (Wieser, 1959) and are always found on locations with high organic inputs (Lambshead, 1986; Riemann, 1968) or otherwise disturbed locations (Bongers et al., 1991). *D. bruciei* is mostly found in association with *Spartina* debris (Bouwman et al.,1984; Warwick, 1981). Bouwman et al. (1984) stated that the non-selective feeding behaviour is efficient if the concentration of food (bacteria) is high and not mixed with non-edible particles of the same size. The decomposing *Spartina* detritus in the mesh containers probably provides high concentrations of bacteria and consequently offers a favourable environment to non-selective deposit feeders. The relation between decomposition rate and the number of the five above-mentioned species depended on the species and on the season. In winter the numbers of the five species did not increase significantly with decomposition rates, but *D. dievengatensis* had significant regression coefficients in spring, summer and autumn, *D. zeelandicus* and *T. acer* in summer and autumn, while *D. bruciei* and *M. parva* increased significantly with decomposition rate in autumn and spring, respectively.

The lack of correlation between the abundances of the majority of the nematode species and decomposition rate, as we found in our study, probably is because many of these species feed on microalgae or diatoms or they are omnivorous or carnivorous species, whereas the microbial organisms directly involved in halophyte decomposition are fungi and bacteria (Newell et al., 1989). The

positive correlation between the abundance of the five microbivorous species and the decomposition rate of *S. anglica* leaves is probably caused by increased microbial biomass production. Higher decomposition rates imply a higher microbial activity and probably also a higher microbial biomass production. This increased availability of food for the microbivorous nematodes allows higher nematode population densities (cf. Findlay, 1982).

The populations of microbivorous species of nematodes on halophyte litter may not only react to changing rates of decomposition, they may also, on their turn, influence these rates. Several studies showed that in the presence of bacterivorous nematodes of the family Monhysteridae higher weight losses of detritus occurred (Findlay & Tenore, 1982; Rieper-Kirchner, 1989; Alkemade et al, 1992). This stimulatory effect of nematodes probably depends on the population density (Tietjen & Alongi 1990; Alkemade et al., 1992). If under field conditions nematodes species are able to stimulate decomposition of *Spartina* leaves then the most likely candidates are those whose numbers correlate with decomposition rate, as were identified in our study. Further investigation on the role of nematodes in decomposition processes should be focused on these decomposition-associated species. The most pronounced effects of these nematode species on decomposition rates may be expected on plant material in the first stages of decomposition in the warmer seasons (spring to autumn), since under these circumstances the highest numbers were found. As the period of the year in which the correlation between numbers and decomposition rate was found varied between species, it is possible that these species exert their effect on litter decomposition in different seasons.

CHAPTER TWO

The population dynamics of *Diplolaimelloides bruciei*, a nematode associated with the salt marsh plant *Spartina anglica*

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ABSTRACT

The halophyte *Spartina anglica* abundantly occurs in the lower parts of Western European salt marshes. *S. anglica* vegetation contains large amounts of standing dead plant material year round. A rich faunal community is present on the decomposing plant parts. *Dipolaimelloides bruciei* is one of the dominant nematode species present on living and dead above-ground parts of *S. anglica* plants. The population dynamics of *D. bruciei* was studied in the field, in relation to the decomposition stage of the plant material. *S. anglica* vegetations are regularly flooded at high tide, which reduces the nematode population density on the plant material, as nematodes are flushed from the plants. The extent of population reduction by flooding was studied in a laboratory experiment. Litter of two different decomposition stages was used.

D. bruciei was present throughout the year on all types of plant material, including living green plant parts. The population densities were highest on the older plant material, where they reached densities of 1000-2000 individuals per g DW. The highest densities were recorded in late summer and autumn.

In the laboratory the rate of removal by flooding was 4.4 times higher on brown leaves than on yellow leaves, while the birth rates were almost identical. As a result the nematode population on yellow leaves increased at a much higher rate than the population on brown leaves and reached much higher densities.

The total number and biomass of *D. bruciei* formed in the *Spartina* vegetation was calculated, assuming that the birth rate of the species depended only on temperature. It was calculated that 9 million of individuals accounting for 114 mg C were formed per m^2 per year. The total amount of carbon ingested by *D. bruciei* as bacterial biomass accounted for 7.5 % of the total bacterial biomass produced. The dominant bacterivorous nematodes together may remove over 20% of the total bacterial biomass.

INTRODUCTION

In the lower zone of West European salt marshes, *Spartina anglica* may form extensive monospecific vegetations. On these sites relatively large amounts of

standing dead plant material is found year-round (Wolff et al., 1979; Groenendijk, 1984). Export of plant-derived detritus from European coastal salt marshes is probably insignificant (Hemminga et al., 1992, 1993). The major part of the dead plant material, therefore, will decompose at the production site. Decomposing leaves remain attached to standing stems for a prolonged period (Newell et al., 1989). Dead plant material of different stages of senescence and decay can be found in the canopy: senescent and dead leaves attached to the still green culms and old, dead, culms with barely any leaves are simultaneously present.

Decomposition is largely a microbial process, but is influenced by a faunal community, which may consist of macro-, meio- and microfaunal species (Swift et al., 1979). In salt marshes especially nematodes are abundantly present in decomposing *S. anglica* litter (Buth & de Wolf, 1985; Hemminga & Buth, 1991). *Diplolaimelloides bruciei* is one of the dominant nematode species present on living and dead *S. anglica* leaves (Bouwman et al., 1984; Warwick, 1981; Hopper, 1970). *D. bruciei* is scarcely found in the surrounding sediment indicating that *D. bruciei* is narrowly associated with *Spartina* litter (Bouwman et al., 1984). In a previous paper, we found that *D. bruciei* can stimulate bacterial decomposition of *S. anglica* leaves under laboratory conditions (Alkemade et al., 1992a). The effect of *D. bruciei* on the decomposition of *S. anglica* is expected to depend on nematode population density and on the total number of nematodes formed, including the individuals lost due to death, flooding etc. The study of population growth and of population density is therefore considered important to assess the potentially stimulating effects of the nematodes under field conditions.

The population density of *D. bruciei* probably depends on at least on three factors: Firstly, since *D. bruciei* is a bacterivorous nematode (Romeyn & Bouwman, 1983; Nicholas, 1984) the population density is expected to depend on the bacterial biomass production; secondly, the population growth rate of *D. bruciei* depends on temperature (Warwick, 1981) and population growth will therefore fluctuate seasonally; thirdly, flooding by seawater is expected to remove nematodes and thus will decrease population densities (c.f. Fleeger et al. 1984).

In this study we investigated the population dynamics of *D. bruciei* on standing living and dead plants of *S. anglica* in a salt marsh of the S.W. Netherlands.

Monthly samples of above-ground *Spartina anglica*, consisting of visually distinguishable stages of decomposition, were collected and the nematode population associated with the plant material was analyzed. The population growth of *D. bruciei* on *S. anglica* litter of different decomposition stages was also studied in laboratory experiments. A facility to simulate flooding was used to assess the influence of flooding on population densities present on the different litter types. Finally, an estimation of the proportion of the bacterial biomass consumed by *D. bruciei* was made.

MATERIAL AND METHODS

Field study

Samples of *S. anglica* were collected in a salt marsh (called Rattekaai), situated in the Oosterschelde, a tidal inlet of the southern North Sea. The samples were taken in a 100 m^2 area near the edge of the salt marsh, where *S. anglica* forms an almost monospecific vegetation. *Salicornia sp.* was also present in very low densities. The elevation of the site was 1.67 m above Dutch Ordnance Level (NAP).

Three paired randomly selected samples were collected monthly from September 20, (1990) to August 15, (1991). Small quadrats (0.04 m^2) were harvested by cutting the plants at the sediment surface. The plant material was put into plastic bags and transported to the laboratory where all samples were divided into 4 different categories: green living biomass; yellow-greenish leaves, with the stem parts bearing these leaves; brown dead leaves, again with the stem parts bearing theses leaves; old brown culms without any leaves. If the lower parts of the stems were covered by a layer of sediment, these parts were discarded, because nematode densities and species composition are highly influenced by this layer (Bouwman et al., 1984). One sample of a pair was dried at 70°C for 48 hours in order to obtain dry weight estimates of the four different categories of *Spartina*. The other sample was fixed in warm 4% formalin to conserve the nematodes.

Nematodes were extracted from the plant material by rinsing the samples with tap water over a house hold sieve (2 mm mesh). Nematodes and other particles

passed through the sieve whereas the coarse plant material was retained on the sieve. The plant material was dried and weighed. The suspension containing the nematodes was further processed using centrifugation as described in Alkemade et al. (1993). In each sample, the total number of nematodes were counted under a dissecting microscope. 100 nematodes were identified and the number and biomass of *D. bruciei* was estimated in one of the three samples of each category collected each month.

Differences between total nematode densities, and between densities of *D. bruciei* on the different categories of *Spartina* material, were evaluated by analysis of covariance. The relations between nematode densities, temperature and flooding frequency were evaluated by regression analysis. Densities were log-transformed prior to analysis. The monthly average air temperature measured at Vlissingen (data obtained from the Royal Dutch Meteorological Institute) and the flooding frequency between sampling days, derived from the high tide levels recorded at the nearby point of Marollegat (data obtained from the Ministry of Transport and Public Works, Tidal Waters Division), were used in the analysis.

Laboratory experiment

Two separate experiments with brown and yellow-greenish leaves were carried out in October/November 1991 and February/March 1992, respectively. Leaves were collected at the Rattekaai salt marsh. The leaves were washed with tap water and dried at air temperature. The leaf material was sterilized by gamma irradiation (2 Mrad) at a facility for food irradiation (Proefbedrijf voor Voedselbestraling Wageningen).

The leaves were cut into 3 cm long fragments and put into small nylon bags with 1 mm mesh. Each bag received ± 0.5 g DW. The bags were soaked in sterile and filtered seawater for about 2 hours. All bags were inoculated with 100 µl of a microbial assemblage. This assemblage was obtained by rinsing *S. anglica* plant material originating from the Rattekaai salt marsh with sterile seawater and filtering the water over a 1.2 µm filter. Specimens of *D. bruciei* obtained from cultures (see Alkemade et al., 1992a), were also added to each bag. The initial nematode population density was determined after two days of incubation.

20 bags were placed in a 1 l glass jar and transferred to an incubator (20 °C). Flooding was simulated four times a week by pumping filtered and autoclaved Oosterschelde seawater into the glass jar. The jar was filled within 30 minutes at a rate of 15 ml min^{-1}. The water remained 2 hours in the jar before it was pumped back into a bottle. The water in the bottle was renewed 2 times a week. Nematode numbers were counted in the water that was removed from the bottle. Two replicate jars were prepared for each experiment. Once a week two bags were removed from each jar. One bag was dried and weighed to determine weight loss. The other bag was fixed in warm formalin (4%) and the number of nematodes was determined. The experiments lasted 42 days.

The population density on the *Spartina* material was assumed to be determined by the rate of removal of nematodes from the litter and the population growth rate. An exponential growth model with a constant rate of removal by flooding was adopted to describe the population dynamics of *D. bruciei*. It was assumed that the death rate was much lower than the birth rate and that the rate of removal by flooding was the only source of disappearance. The model can be described by the following equation:

$$N_t = N_0 e^{(\beta-\alpha)*t} \qquad (1)$$

where N_t is the number of nematodes present on the *Spartina* detritus at time t, N_0 the number of nematodes present at the start of the experiment, ß is the birth rate and α is a parameter expressing the rate of flushing of nematodes due to flooding. The flooding parameter can be estimated, independently of ß, by using the number of nematodes flushed away and the number of nematodes present on the leaves. As the days on which the bags were removed from the jar did not coincide with the days on which the flushing took place, the number of nematodes present on the leaves at the moment of flushing were calculated by linear interpolation between the sampling days. The parameter α was found by linear regression of the number of nematodes flushed away per unit time on the number of nematodes present on the *Spartina* leaves. The log-likelihood function of the Poisson distribution was used in the estimation procedure, as the data were counts (McCullagh & Nelder, 1989). Using the calculated values for α, the birth rate ß was then calculated by nonlinear regression of the exponential

growth equation (1). Again the log likelihood function of the Poisson distribution was used. Subsequently, the total numbers developed during the course of the experiments were estimated by integrating the growth over time:

$$P = \int_0^T bN dt \qquad (2)$$

where P denotes the total numbers of nematodes and T denotes the last day of the experiment.

The regression analyses were carried out using SYSTAT 5.0 (Wilkinson, 1990) and the total number of nematodes formed was calculated by numerical integration, using SENECA 1.5 (De Hoop et al., 1992).

RESULTS

Field study

Green biomass of *S. anglica* showed a clear seasonal pattern (Figure 1). In August a maximum of 850 g DW per m^2 of green biomass was reached. Green *S. anglica* leaves were found year-round, but in winter months only a very low biomass was observed. Yellow leaves were also found in most of the samples. The peak amount of 200 g DW per m^2 of yellow leaves was found in January. Older plant material of the categories "brown" and "old stems" were found throughout the year in relatively constant amounts. The total dead biomass, consisting of the categories "yellow", "brown" and "old stems", exceeded the biomass of the green plant parts in every month, except in August.

The total nematode densities, all species included, expressed as numbers per g DW of plant material are shown in Figure 2. The numbers found on brown and old material were usually higher than on yellow and green leaves and showed little seasonal variation. The numbers fluctuated between 1,000 and 4,000 individuals per g DW. The numbers of nematodes on green and yellow plant parts were usually much lower, but showed a sharp peak in March and June,

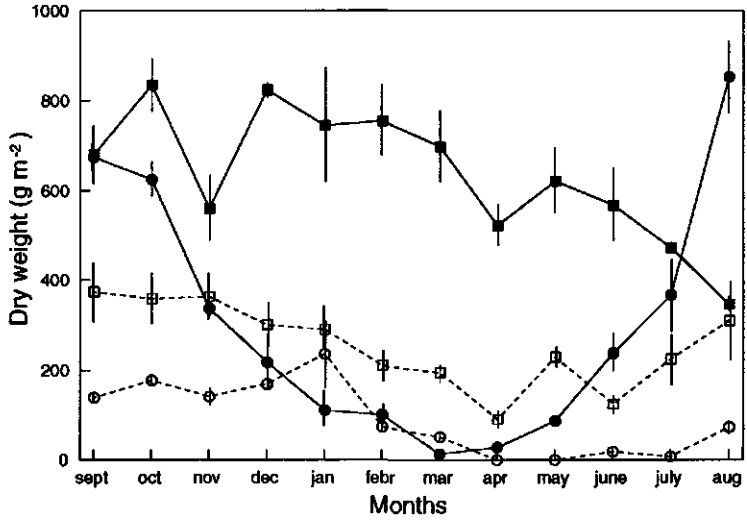

Figure 1: Dry weights (g/m²) of four categories of standing *S. anglica* plant material in the salt marsh (● = living green biomass, ○ = yellow leaves, ■ = brown leaves, □ = old stems) (means and standard errors, n=3).

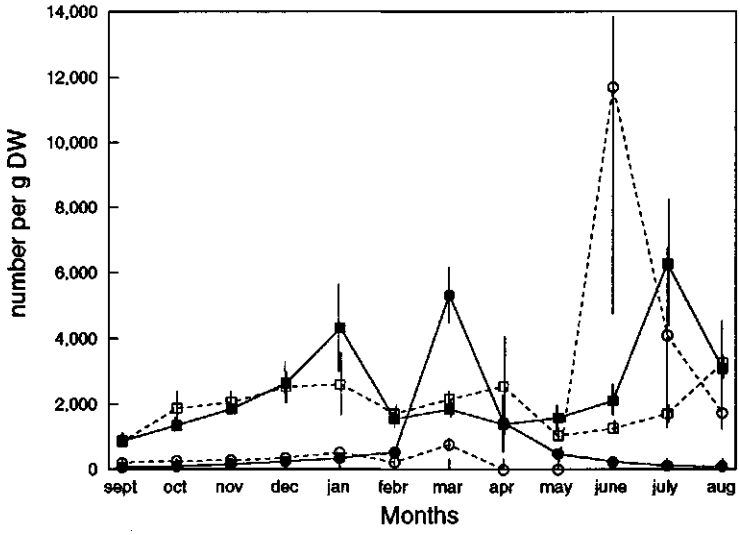

Figure 2: Total nematode densities (number / g DW) on four categories of standing *S. anglica* plant material. (● = living green biomass, ○ = yellow leaves, ■ = brown leaves, □ = old stems) (means and standard errors, n=3).

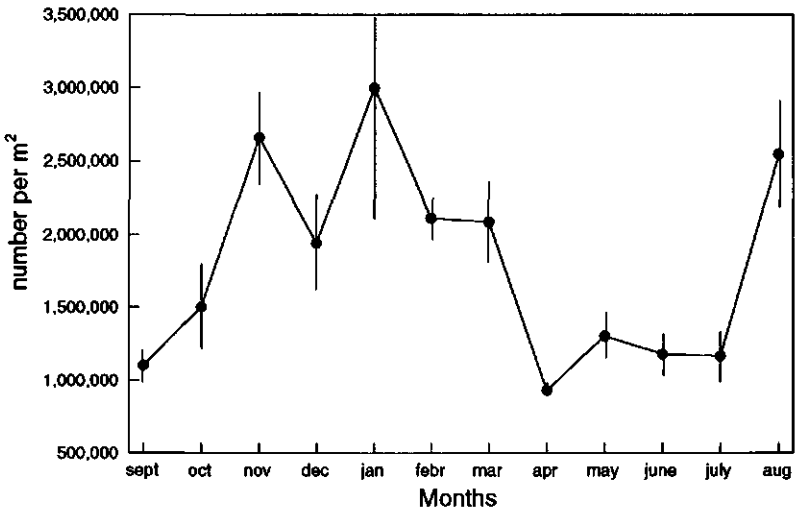

Figure 3: Total number of nematodes (number / m^2) on above-ground plant parts of *S. anglica* plants (means and standard errors, n=3).

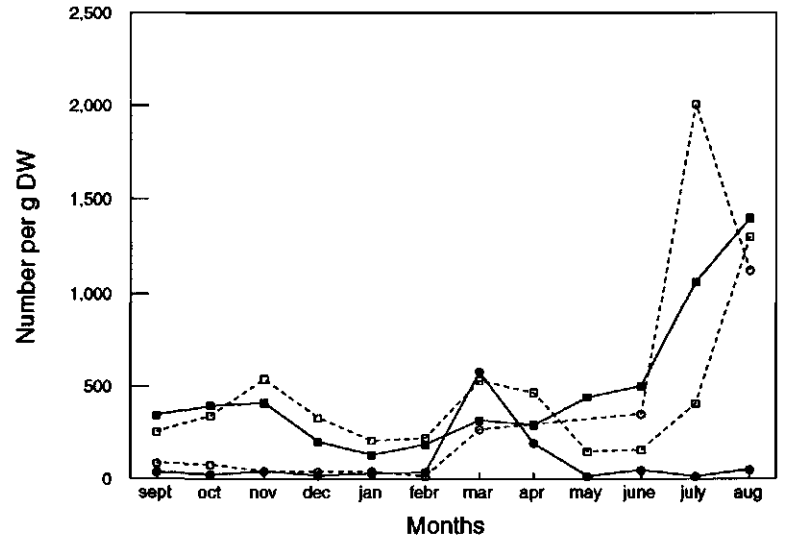

Figure 4: Densities of *D. bruciei* on four categories of standing *S. anglica* plant material. (● = living green biomass, ○ = yellow leaves, ■ = brown leaves, □ = old stems).

respectively. The total numbers per m^2 of *Spartina* vegetation fluctuated from about 1 million in spring and summer to 2 to 3 million in autumn (Figure 3).
D. bruciei was found in all samples. Densities differed significantly between the various litter categories ($P < 0.05$). On the brown plant parts and the old stems, population densities were usually higher than on the green and yellow plant parts (Tukey HSD: $P < 0.05$). Population density on the green leaves was usually lower than 100 per g DW, but a much higher density was found in the March sample (Figure 4). On yellow leaves the population density was usually about 100 per g DW, but higher population densities were found in the summer reaching a maximum of more than 2,000 individuals per g DW in July. On brown plant parts population densities were highest in summer (July and August), and reached densities of 1,400 individuals per g DW; in the other seasons the numbers of *D. bruciei* were much lower. On old stems a similar pattern was observed: in summer the highest numbers were found. In September and October 1990 and in August 1991 *D. bruciei* formed 40 - 50 % of the total nematode community on the *Spartina* plants. In the other months *D. bruciei* did not dominate: during the winter months less than 10 % of the total number belonged to this species.
Regression analyses showed that the log-nematode densities were not related to the flooding frequency. The log-number of nematodes on yellow and on brown leaves were highly correlated with the mean monthly temperature ($R^2 = 0.72$ and 0.80, respectively). On green plant parts and old stems no correlation was found between the nematode densities and the temperature.

Laboratory study

During the experiments the weight of *Spartina* leaves in the litterbags decreased gradually. The weight losses from the yellow leaves were higher than from the brown leaves. After 42 days the yellow leaves lost, on average, 32 % of the initial weight, whereas the brown leaves lost less than 10 %.
The initial nematode population on the yellow leaves was smaller than on the brown leaves. After two days of incubation, at the first sampling day, 25 nematodes g^{-1} DW were found on the yellow leaves and 300 individuals on the brown leaves.

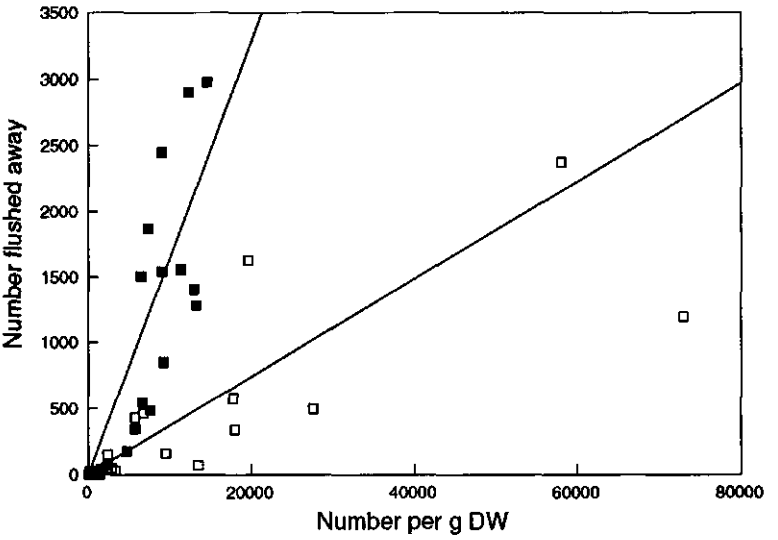

Figure 5: Number of individuals of *D. bruciei* flushed away from yellow leaves (□) and from brown leaves (■) of *S. anglica* plotted against the number of individuals present on decomposing leaves.

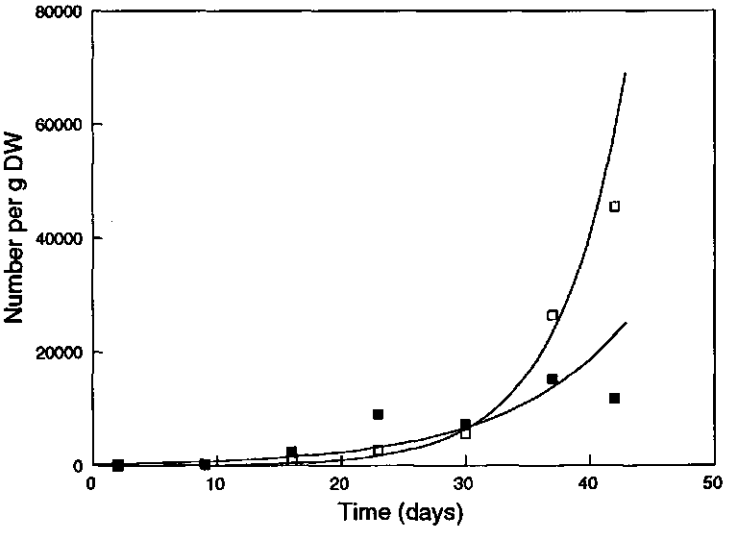

Figure 6: Population growth of *D. bruciei* present on *S. anglica* leaves. Fitted lines are exponential growth curves. (□ = yellow leaves, ■ = brown leaves).

These figures were used in the calculation of the population growth parameters. Conspicuously more nematodes were flushed away from the brown leaves, relative to the numbers in the litter bags, than from the yellow leaves. In Figure 5, the numbers flushed away during one flushing time isplotted against the numbers present on the leaves. The flooding parameter α was 4.4 times higher on brown leaves than on yellow leaves, 0.164 and 0.037 per flushing time, respectively. Apparently α depended largely on litter type. These figures and the initial population densities were used to calculate the birth rate ß. Since the flooding parameter α was calculated as a rate per flushing time and flushing occurred 4 times a week, these parameters had to be transformed to rates per day by dividing the parameters by 7/4. In figure 6 the growth curves of the populations on yellow and on brown leaves are shown. The prolonged initial phase of population growth on yellow leaves is due to the lower initial population density. At the end of the experiment much higher densities were reached on yellow leaves than on brown leaves. The calculated birth rates in both populations were almost identical: 0.206 (± 0.008, 95 % confidence limits) on yellow leaves and 0.197 (± 0.01, 95 % confidence limits) on brown leaves. The calculated total number of nematodes formed was 55,800 on yellow leaves and 42,000 on brown leaves. The difference between these two figures was low in comparison with the population densities present at the end of the experiment, ± 56,000 on yellow leaves and ± 11,500 on brown leaves.

DISCUSSION

The *Spartina anglica* vegetation in the Rattekaai salt marsh contains a large proportion of dead plant material. Almost year-round more dead plant material was found than green living plant material. Nematodes were present throughout the year on green plant parts as well as on senescent and decaying plant parts. The highest numbers of nematodes per m^2 were found during the autumn and winter, coinciding with the highest amounts of dead plant material present on the salt marsh. The total numbers of nematodes ranged from $1*10^6$ to $3*10^6$ per m^2. These numbers are of the same magnitude as the numbers found in salt marsh and estuarine sediments: the total number in the sediment of a *S. anglica*

vegetation varied from 2 to 10 million individuals per m^2 (Alkemade et al. submitted), and in coastal sediments usually densities between 1 and 20 million individuals per m^2 are observed (e.g Heip et al., 1985; Warwick & Price, 1979; Teal & Wieser, 1966). Thus, the nematode community living on the standing dead plant parts forms a substantial part of the total salt marsh nematofauna.

The bacterivorous nematode *Diplolaimelloides bruciei* is one of the dominant species present on the above-ground plant parts, representing up to 50% of the nematodes in late summer and autumn. Bouwman et al. (1984) found similar percentages for *D. bruciei* on *S. anglica* vegetation in the north-eastern part of the Netherlands. The numbers of *D. bruciei* found in the field were much lower than the densities observed under laboratory conditions, which possibly is due to the more moderate conditions in the laboratory. In the field, temperature fluctuates much more than in the laboratory and usually does not reach values over 20°C. Flooding is irregular and probably often more vigorous than in the laboratory experiment, causing losses of nematodes from the population.

In the laboratory experiment the population dynamics of *D. bruciei* could be described by an exponential growth model, consisting of two components: a constant birth rate and a constant rate of disappearance of nematodes as a result of flushing. The results showed that the birth rates were almost identical on both leaf types (0.206 and 0.197 on yellow and brown leaves respectively) and were close to the value of 0.21 d^{-1} found by Warwick (1981) for the rate of population increase of the same species at 20 °C. The present values are also consistent with our earlier estimate of 0.22 d^{-1} (Alkemade et al., 1992a). The growth of the nematode population on the different leaf types was mainly affected by flooding. On brown leaves the proportion of nematodes flushed away relative to the population density present on the leaves was 4.4 times higher than on the yellow leaves. This may be caused by the fact that the surface properties of the leaf litter alters with age. During decomposition the leaves disintegrate to tangles of long shreds (Newell et al. 1989). At the beginning of the experiments both leaf types had clear surface structures consisting of longitudinal grooves. Nematodes may be relatively protected against the risk of being flushed away in these grooves. The brown leaves lost this surface structure within a few weeks. As a result, nematodes may loose their "grip" and consequently are flushed away during high tide. The structure of the yellow leaves, in contrast, remained

much more intact during the experiment, which may have enabled the nematode population to reach relatively high densities.

The disappearance of large numbers of nematodes from disintegrating leaves is not necessarily merely a loss to the population. Some of these nematodes may reach other *Spartina* sites. We observed that *D. bruciei* remained in suspension for several hours, sufficiently long to migrate within a salt marsh during high tide.

In the field the numbers of *D. bruciei* were usually highest on the older plant litter of the categories "brown" and "old stems". Only in late summer the numbers found on yellow leaves exceeded the numbers on brown leaves and old stems. During the first phases of decomposition the population density apparently increases from the low numbers on yellow leaves to the high numbers on brown leaves. Probably, as discussed above, the numbers decrease again as the leaves gradually loose their surface structure. In the laboratory experiments the nematode population reached much higher densities on the (initially) yellow leaves than on brown leaves, since the yellow leaves had changed into "brown" leaves at the end of the experiment, whereas the initially brown leaves lost their leaf-surface structure in the course of the experiment, resulting in large losses during flooding. On the stems the changes in population density is probably slower, since the stems disintegrate at a much slower rate.

With the data available it is possible to make an estimation of the total number and biomass of *D. bruciei* formed on a *Spartina* vegetation and of the bacterial biomass consumed by the nematodes. The total amount of *S. anglica* detritus formed approximately equals the total yearly production of the macrophyte. The peak above-ground living biomass can be considered as a rough estimate of the yearly production of *S. anglica* (De Leeuw et al., 1991). The peak above-ground living biomass at the Rattekaai salt marsh occurred in August, being 850 g DW per m^2, equivalent to ± 340 g C per m^2. The nematode production depends on the number of nematodes present at the sampling time and the growth rate of the nematodes (Heip et al., 1982):

$$P=\sum r_t N_t \qquad (3)$$

where P is production, r_i the growth rate and N_i the number of nematodes in the i-th sampling interval. Warwick (1981) showed that the growth rate depended on temperature and derived a linear regression formula for the relation between the birth rate and the temperature. Using this formula the average monthly growth rate of every month was calculated from the mean monthly temperatures. In table 1 the mean monthly temperature, the calculated growth rates, the total number of *D. bruciei* per m^2 and the estimated daily total number of nematodes formed are shown. An estimate of the total number of *D. bruciei* formed during the year was obtained by summing the daily production of new nematodes, and was approximately 8.89 million individuals. The mean biomass per individual calculated each sampling day (table 1). The total biomass production, calculated from these figures was approximately 1.07 g wet weight, this is equivalent to 114 mg C per m^2, which represents only a small fraction of the *S. anglica* production. The amount of bacteria ingested by the nematodes is much higher than the biomass produced, since a large amount of bacterial C ingested by the nematodes is lost by defecation, excretion and respiration. The fraction of the bacterial C ingested which is transferred to biomass of *D. bruciei* is not known. Herman & Vranken (1988) gave estimates of the assimilation efficiency and the production efficiency of the related species *Monhystera disjuncta*. When we assume that the efficiency of *D. bruciei* is similar to that of *M. disjuncta*, the fraction of bacterial C transferred into nematode biomass can be considered as the product of the assimilation efficiency, and the production efficiency and equals approximately 0.15. Thus, to form 114 mg C of nematode biomass, 760 mg C of bacterial biomass is needed.

If *Spartina* carbon is transformed into bacterial biomass with an efficiency of 10 %, the maximum total bacterial biomass formed is equal to 34 g C per m^2 per year. This figure, however, is unrealistically high, since only a part of the *Spartina* detritus is decomposed by bacteria. Another part is decomposed by fungi or is removed from the salt marsh by the tides. Padgett et al. (1985) estimated that about 30 % of *S. alterniflora* leaves were decomposed by bacteria. The total bacterial biomass formed may be equivalent to 10 g C per m^2 per year. Thus the bacterial biomass ingested by *D. bruciei* may account for 7.5 percent of the total bacterial biomass formed during decomposition of *Spartina* litter. *D. bruciei* was not the only bacterivorous nematode present on *S. anglica*

leaves. The numbers of *Monhystera disjuncta* and *Pellioditis marina* were of the same order as the numbers of *D. bruciei*. If equal growth is assumed for the three bacterivorous nematodes, the ingested proportion of bacterial biomass may be over 20 %.

Table 1: Estimation of monthly production of *D. bruciei*. For calculation of the monthly production of nematodes expressed as mg C per m^2, a C-content of nematodes of 10.6 % of wet weight was assumed (Heip et al. 1985).

Month	mean temp °C (d^{-1})	growth rate (µg)	number m^{-2}	wet w. per ind	monthly production number of individuals	monthly production (mg C m^{-2})
Sept 90	14.7	0.13	303700	0.144	1184400	18.1
Oct 90	13.1	0.11	221800	0.156	722100	11.9
Nov 90	7.8	0.04	390700	0.130	439700	6.1
Dec 90	5.1	0.001	136300	0.126	5500	0.1
Jan 91	4.1	0.0	102100	0.130	0	0.0
Febr 91	0.5	0.0	163100	0.117	0	0.0
Mar 91	8.1	0.04	231600	0.222	288600	6.8
Apr 91	8.8	0.05	153100	0.184	233900	4.6
May 91	10.0	0.07	177500	0.184	356800	7.0
June 91	13.1	0.11	207700	0.133	676200	9.5
July 91	18.2	0.18	247800	0.118	1314700	16.4
Aug 91	18.8	0.18	662200	0.086	3673700	33.5
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Total					8895700	114.0

CHAPTER THREE

Path analyses of the influence of substrate composition on nematode numbers and on decomposition of stranded seaweed at an Antarctic coast

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ABSTRACT

Large amounts of seaweed are deposited along the coast of Admiralty Bay, King George Island, Antarctica. The stranded seaweed partly decomposes on the beach and supports populations of meiofauna species, mostly nematodes. The factors determining the number of nematodes found in the seaweed packages were studied. Seaweed/sediment samples were collected from different locations, along the coast near Arctowski station, covering gradients of salinity, elevation, proximity of penguin rookeries. On the same locations decomposition rate was determined by means of permeable containers with seaweed material. Models, including the relations between location, seaweed and sediment characteristics, number of nematodes and decomposition rates, were postulated and verified using path analysis. The most plausible and significant models are presented.

The number of nematodes was directly correlated with the height of the location, the carbon to nitrogen ratio, and the salinity of the sample. Nematode numbers apparently were indirectly dependent with the sediment composition and water-content. We hypothesize that the different influences of melt water and tidal water, which affect both salinity and water content of the deposits, are important phenomena underlying these results.

Analysis of the relation between decomposition rate and abiotic, location-bound characteristics showed that decomposition rate appeared to be dependent on the water content of the stranded seaweed and the sediment composition. Decomposition rates were high on locations where water content of the deposits was high. The running water from melt water run-off or from the surf probably increased seaweed weight losses in these situations.

INTRODUCTION

Along the coast of Admiralty Bay, King George Island (Antarctica), an extensive vegetation of macroalgae occurs. The production of these algae is high in summer and comparable to the production of laminarians in temperate regions (Dieckman et al., 1985). The macroalgal community of Admiralty Bay is composed of about 18 species of which the red algae *Leptosomia simplex* and

Iridaea obovata and the brown algae *Desmarestia* sp. and *Himantothallus grandifolius* are predominant (Zielinsky, 1981).

The growth of macroalgae occurs at the base of the plants, while erosion and decomposition take place at the thallus tips (Dieckmann et al., 1985). Fragments of the thallus are removed by storms, tidal currents and ice movements. Large amounts of algal debris are washed ashore. A substantial proportion of this material is returned to the surf by successive high tides, but the remainder accumulates along the coast in packages which are often covered by sediment (Inglis, 1989; Zielinski, 1981).

Stranded algal debris often decomposes very quickly. On a sandy beach in South Africa Koop et al. (1982a) found that within 8 days the weight of algal-debris decreased with 73-77 %. A small amount of the organic matter was consumed by macrofauna, but over 90 % was mineralized by bacteria (Koop et al.,1982b).

In temperate regions algal deposits support large amounts of amphipods, insects and other macrofauna (Griffiths & Stenton-Dozey, 1981; Bedford & Moore, 1984). Most of the macrofaunal species are herbivores and fragmentate the seaweed detritus (Inglis, 1989). Nematodes and other meiofaunal groups found in seaweed deposits are mainly bacterivores and may be of considerable importance in reworking kelp debris (Inglis, 1989). Their activity may influence the bacterial decomposition of seaweed (Rieper-Kirchner, 1989).

In this study it was attempted to find factors which determine the number of nematodes found in seaweed deposits along the coast of Admiralty Bay. Seaweed characteristics and site related factors might influence the number and composition of the nematode community. Therefore we sampled on different sites covering gradients of salinity, proximity of penguin rookeries and heights of the deposits. On the same sites the decomposition rate of seaweed was studied in relation to location characteristics. Possible relations between nematode numbers, seaweed decomposition and other factors are discussed. Path analysis (Li, 1975) was used to construct possible causal relations between the measured variables.

MATERIAL AND METHODS

Collection of samples

From 15 to 18 december, 1990, seaweed deposits were sampled along the 2.35 km long coast-line near Arctowski station between Point Thomas and the Ecology Glacier (Fig. 1). On this part of the Admiralty Bay-coast large amounts of seaweed debris are washed ashore (Zielinsky, 1981). The coastline was divided into 4 regions with different characteristics. The first region, from Point Thomas to Shag Point, is a dynamic region. Large amounts of seaweed debris are washed ashore, but return to the sea within a few days. Generally, only few penguins are present on this part of the beach. The second region is a more sheltered beach between Shag Point and the Penguin rookeries at Rakusa Point. The seaweed deposits are situated higher on the shore and on several places the seaweed is covered by sediment. High numbers of penguins rest here. The third region is located under the penguin rookeries at Rakusa Point. Again high numbers of penguins are present; the locations are situated high on the shore and on several places fresh water streams debouch, crossing the seaweed deposits. The fourth region is a sheltered tidal sandflat near Ecology Glacier. Melt water from the glacier runs over the sandflat and cross the seaweed deposits. Low numbers of penguins are present. Differences of seaweed- and site characteristics between the four regions were verified by use of discriminant analysis (Christensen, 1990).

In each region 5-8 seaweed deposits were randomly selected, so that in total 26 sites were sampled. On most of the sites the seaweed was mixed with sediment. On every site, two random samples of 100 - 200 g wet weight were collected. After weighing, one of the samples was fixed in warm formalin (4%), to extract and conserve meiofauna, the other was dried at 70 °C for 24 hr prior to chemical analyses and determination of sediment composition. The seaweed species involved, were not determined.

The height differences between the sampled sites were determined, using a water-level. The flood mark was used as a reference, assuming that the flood mark was at the same height along the entire coast-line under study.

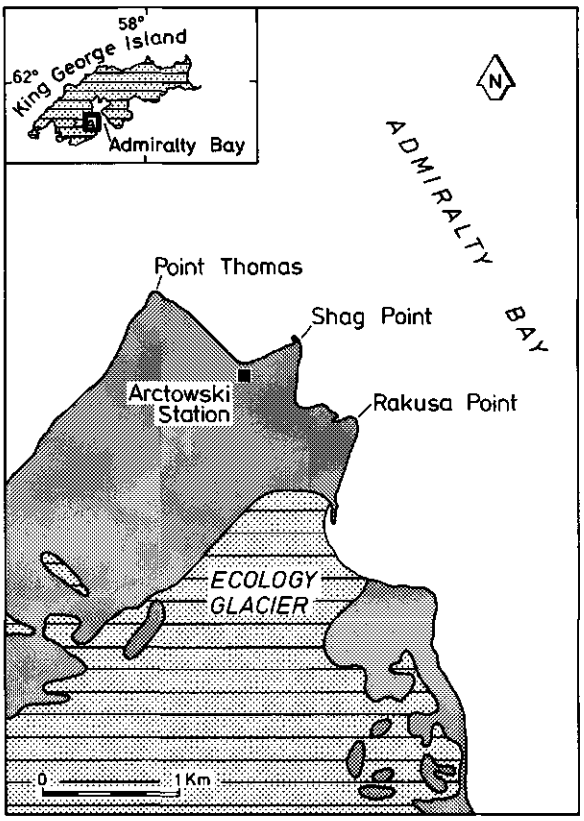

Figure 1: Map of the coastal region near Arctowski Station, King George Island.

Meiofauna

Meiofauna was extracted from the 26 fixed seaweed samples in the laboratory at Arctowski Station. The samples were rinsed with an excess of tap water through a house-hold sieve (2 mm mesh) to remove the coarse material. The suspension containing the meiofauna was passed over a series of four 45 µm mesh sieves. The debris retained on the sieves was collected in 250 ml centrifugation tubes with tap-water. After centrifugation (5 min. at 3000 rpm) the supernatant was discarded and the meiofauna in the pellet was extracted by two repeated centrifugation steps (1 min) with a $MgSO_4$ solution (specific gravity of 1.28).
The nematodes were counted, processed with the Seinhorst-method and mounted in mass slides. The mass slides were prepared as follows: with a stamp a square

of parrafin wax was placed on a slide (50*100 mm), a drop of glycerin with nematodes was placed in the centre of the slide and covered with a coverslip (45*45 mm). The slide was heated till the parrafin melted. This method facilitates identification of a large number of specimens in each sample and is developed at the Nematology department of the Agricultural University, Wageningen, The Netherlands. From each sample 100 specimens were identified.

Chemical analysis

The 26 samples collected for chemical and physical analyses were processed in the laboratory of the Netherlands Institute of Ecology. The samples usually were a mixture of seaweed debris and sediment and will therefore be referred to as seaweed/sediment samples. Sediment grains larger than 2 mm in diameter, referred to as coarse sediment fraction, were first removed from the samples and weighed. The samples were ground and a subsample was used for determination of ash-free dry weight (AFDW). We assumed that the ash content of the samples was determined by their fine sediment fraction.
Total carbon and nitrogen were determined with a Carlo Erba Nitrogen/Carbon analyzer, type: NA 1500. Phosphate content was determined by colorimetric analysis. Chlorinity of the water fraction of the sample was determined by potentiometric titration.

Decomposition

Decomposition rate of seaweed was measured using permeable containers, i.e., segments of plexiglass cylinders with an inner diameter of 7 cm and a height of 2 cm, with both ends closed with 1 mm mesh gauze.
Seaweed debris of *Iridaea* sp., freshly washed-ashore, was collected, washed with tap water and the surface was dried with a tissue. The containers were filled with 15 g fresh weight of seaweed. Another portion of the collected seaweed was dried and weighed in order to calculate the relation between wet weight, dry weight and ash-free dry weight. On each of the 26 sampling sites (see above) one container was placed and covered by seaweed. The containers were collected after 20 days and the contents were subsequently dried at 70°C

for 48 hr. Dry- and ash-free dry weight were determined. Decomposition rate was expressed as percentage ash-free dry weight loss. During the experiment the temperature ranged from -5 °C to +10 °C.

Statistical analysis

Following the procedure outlined by Schwinghamer (1983) we used path analysis to construct possible causal models consisting of location, seaweed and sediment characteristics as independent variables and the number of nematodes and decomposition rate, respectively, as dependent variables. All but one of a group of independent variables, showing multicollinearity were rejected. Biological plausible models were postulated and verified with the data. The structure of the model was based on the individual significance of regressors, the coefficient of determination (R^2) of the different dependent variables and the comparison of correlation coefficients (r_{calc}) calculated by means of path analysis and the observed Pearson correlation coefficient (r_{obs}) from the data set.
Path coefficients (p_{YA}), the numerical value associated with the cause-effect relationship between variables A and Y, were estimated by least-squares regression. The path coefficients are the standardized partial regression coefficients and represent the direct components of correlation between variables (Li, 1975). Path diagrams were constructed in order to visualize the direction and weight of the relations between all variables in the models. Correlation coefficients (r_{calc}) among variables indicated by a given path diagram were calculated according to the general formula:

$$r_{ay} = \sum_{q} p_{yq} r_{aq}$$

where a and y are 2 variables; q is the index running over all variables from which paths lead directly to variable y (Schwinghamer, 1983; Li, 1975).
SYSTAT (Wilkinson, 1990) was used for calculation of correlation coefficients (r_{obs}) and regression models. The regression output provides the standardized partial regression coefficients, p-values and the coefficients of determination (R^2) of the dependent variables. A path diagram was constructed by combining the regression models used.

RESULTS

Meiofauna

Nematodes were the dominant group of meiofauna in the samples. Only in a few samples low numbers of other meiofauna groups, such as copepods and tardigrades, were found. On one location a high number of a collembolan species was found. This location was situated near a penguin rookery, where the influence of seawater was low and the seaweed detritus consisted only of some holdfasts.

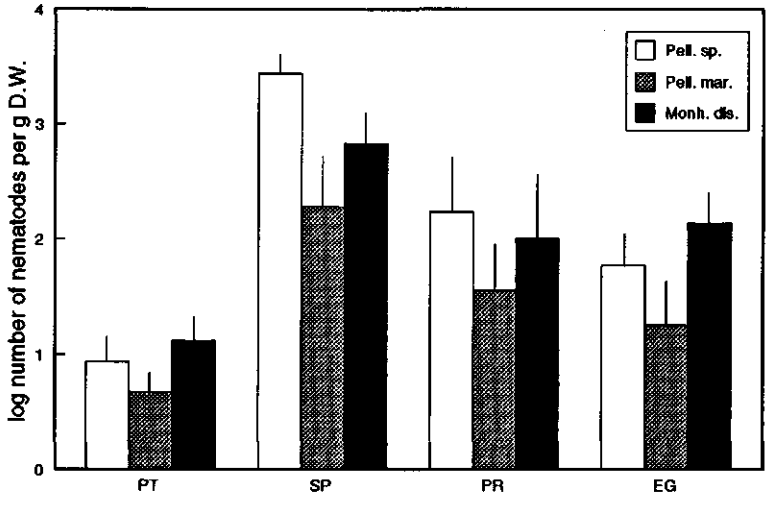

Figure 2: Mean abundances of the three dominant nematode species, *Pellioditis* sp. *Pellioditis marina* and *Monhystera disjuncta*, occurring in seaweed deposits in the four different coastal regions (The four regions are abbreviated: PT = region between Point Thomas and Shag Point, SP = region between Shag Point and Rakusa Point, PR = region near penguin colony on Rakusa Point and EG region near Ecology Glacier). Bars indicate 1 standard error.

Eight species of nematodes were found in the seaweed/sediment samples, but only three species were found in nearly all samples. Of these, two species were of the genus *Pellioditis* and one was the monhysterid *Monhystera disjuncta*.
Nematode numbers varied much among the different locations. On some locations only a few specimens per g of substrate were found, but the maximum

number was 26,000 specimens per g of substrate. Mean numbers of the three dominant nematode species in the four regions are shown in figure 2.

Seaweed, sediment and location characteristics

Table 1 shows the values of seaweed, sediment and location characteristics, at the sample locations. The data were used in a discriminant analysis in order to verify the division into the different regions. Overall differences between the regions were significant (Hotelling-Lawley trace = 5.578, $P < 0.001$). Two locations of the Penguin Rookery region were classified in other regions. One of these was more related to the region between Shag Point and Rakusa Point, the other to the Ecology Glacier region.

Decomposition

Decomposition rates varied much among the different sampling locations. Mean decomposition rate, as was calculated over all locations, was 35 % AFDW-loss within 20 days. On some locations no weight loss took place; in contrast on one location 87 % of the seaweed disappeared within 20 days. Decomposition rates were highest in the penguin-rookery region and near the Ecology Glacier (52 % in 20 days, on average), and in the region near Point Thomas (49 %). The lowest decomposition rates were found in the region between Shag point and Rakusa point (14 %).
On three locations in the Point Thomas region the containers and the entire seaweed deposits were carried away with the tides. After 20 days one location near the penguin rookeries was not accessible anymore because of increased flows of melt water.

Path diagram

Multicollinearity was found between Carbon, Nitrogen and Phosphate contents and C:N ratio. Furthermore Carbon and Nitrogen contents were highly correlated with the fine sediment fraction. We choose C:N ratio and the fine sediment fraction to represent these groups of variables, firstly since these two variables

Table 1: Location and seaweed/sediment characteristics on the 26 sampled locations. The variables are abbreviated: RH = Relative Height (cm), CSF = Coarse sediment fraction (% of DW), FSF = Fine sediment fraction (% of DW), CHC = Chloridity g/l water), WC = Water content (% of wet weight), NIT = Nitrogen content (% of DW), PHO = Phosphate content (% of DW), CAR = Carbon content (% of DW), C:N = C:N ratio. (Abbreviations of region names see figure 2).

		RH	CSF	FSF	CHC	WC	NIT	PHO	CAR	C:N
PT	1	65	3.1	38	19	84	3.2	0.7	28	8.7
	2	65	1.5	32	21	78	2.9	0.6	30	10.2
	3	65	2.9	29	22	80	3.0	0.6	33	11.0
	4	0	7.3	33	25	67	2.5	0.4	27	10.6
	5	75	6.7	27	30	64	3.5	0.4	33	9.3
	6	70	0.1	25	20	72	2.9	0.4	34	11.7
	7	90	0.1	27	17	77	2.9	0.5	37	12.8
mean		61	3.1	30	22	75	3.0	0.5	32	10.6
SP	1	135	9.4	35	16	76	3.2	0.8	30	9.3
	2	165	0.0	24	13	72	4.4	1.0	39	8.9
	3	130	8.7	76	24	53	1.0	0.4	7	7.5
	4	150	30.8	27	12	69	1.7	0.4	22	12.8
	5	110	4.9	45	18	72	3.1	0.7	25	8.1
	6	105	25.6	47	2	51	2.2	0.4	17	7.6
	7	110	0.8	61	22	41	1.8	0.4	19	10.7
	8	90	3.3	40	8	57	3.5	0.5	34	9.7
mean		124	10.4	44	14	61	2.6	0.6	24	9.3
PR	1	115	3.2	61	17	72	2.0	0.4	16	8.0
	2	115	14.0	38	25	64	2.5	0.5	24	9.7
	3	100	0.0	20	1	87	4.3	0.6	44	10.1
	4	240	0.4	25	0	77	3.6	0.6	40	11.2
	5	200	0.5	53	0	76	2.2	1.0	22	10.1
mean		154	3.6	39	9	75	2.9	0.6	29	9.8
EG	1	130	1.0	39	4	79	3.0	0.4	29	9.4
	2	115	0.2	37	0	77	1.7	0.4	27	16.3
	3	70	0.0	19	1	84	3.3	0.4	38	11.5
	4	60	0.0	52	16	80	2.4	0.4	21	8.9
	5	95	6.8	15	0	83	3.9	0.5	37	9.5
	6	30	0.0	32	3	90	3.0	0.4	31	10.4
mean		84	1.3	32	4	82	2.9	0.4	31	11.0

were not correlated and secondly because C:N ratio is a frequently used parameter for identifying the nature of organic matter.

Path analysis of the factors likely to affect nematode numbers led to the model illustrated as a path diagram in figure 3. The model presented is the end result of a process of optimization wherein many combinations of variables and, biologically plausible, relationships were examined. The correlation coefficients of the model are shown in table 2. The regression equations calculated from the analysis and the corresponding coefficients of determination are shown in table 3.

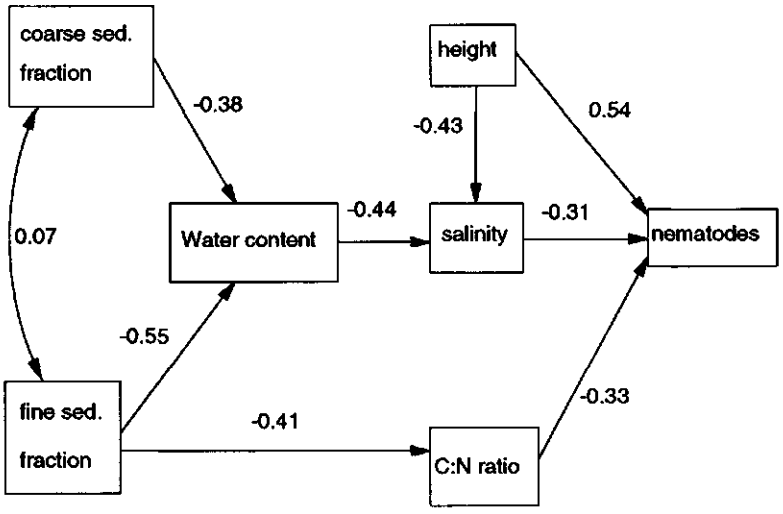

Figure 3: Path diagram resulting from path analysis with log number of nematodes as dependent variable.

In the model (Figure 3) nematode number is directly influenced by the carbon to nitrogen ratio, the salinity and the relative height of the sampled site. An indirect path from relative height via salinity to nematode number was also observed. The C:N-ratio was influenced by the low sediment fraction. Salinity depended on the water content of the sample and the water content was influenced by the sediment composition. The sediment composition and the water content had only indirect influences on the nematode number. The correlation coefficients calculated from the path diagrams estimated the observed correlation coefficients quite well. Only the observed correlations between coarse sediment fraction and nematode number and the correlation between

water content and nematode number were much higher than the calculated correlations.

Table 2: Observed and calculated correlation coefficients between log number of nematodes and independent variables.

	r_{obs}	r_{calc}
Relative height (cm)	0.66	0.67
Coarse sediment fraction (% of DW)	0.33	0.05
Fine sediment fraction (% of DW)	0.15	0.13
Chloride content ($^0/_{00}$ in Water)	-0.43	-0.43
Water content (% of wet weight)	-0.18	-0.01
Phosphate content (% of Substrate)	0.35	-
C:N ratio	-0.25	-0.25

Table 3: Multiple regression models for model I used to construct the path diagram (see figure 3)

log(Nematodes) = 3.701 + 0.011*Rel.Height - 0.034*chloridity - 0.178*C:N ratio	$R^2 = 0.57$
Chloridity = 47.321 - 0.081*Rel.Height - 0.359*Water content	$R^2 = 0.33$
C:N ratio = 12.125 - 0.054*Fine sed. fraction	$R^2 = 0.17$
Water content = 91.477 - 0.439*Fine sed. fraction - 0.568*Coarse sed. fraction	$R^2 = 0.47$

A separate path analysis of the factors affecting the decomposition rate was carried out as well. We related decomposition rate, expressed as the weight loss of seaweed in containers during 20 days after sampling, only to the relatively stable location-bound characteristics. Biotic characteristics, such as carbon- and nitrogen contents and nematode number, might change within a few weeks.

Since we measured the biotic characteristics only at the beginning of the incubation of the containers, they were not included in the analysis. Figure 4 shows the path diagram resulting from path analysis. According to the model, decomposition rate is influenced by the water-content of the seaweed deposit and the coarse sediment fraction. An indirect path from the fine sediment fraction via water content to the decomposition rate was observed. The relative height and the salinity did not affect the decomposition rate or one of the other variables. The observed and calculated correlation coefficients of the model are shown in table 4. The regression equations calculated from the analysis and the corresponding coefficients of determination are shown in table 5.

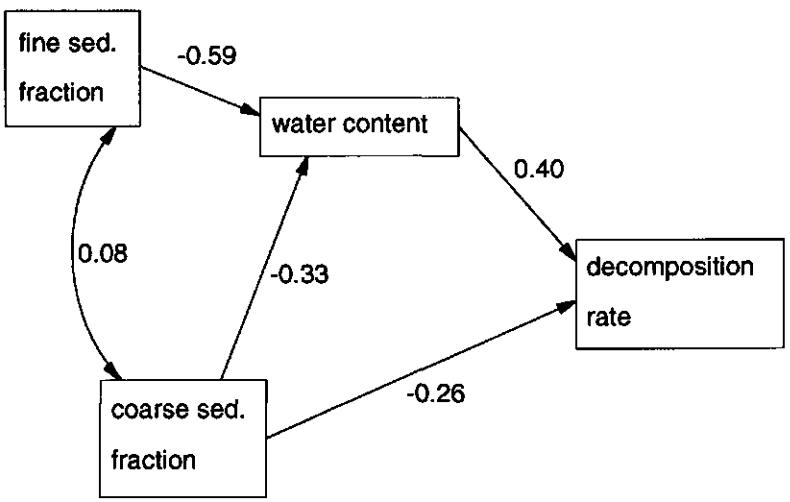

Figure 4: Path diagram resulting from path analysis with decomposition rate as dependent variable.

DISCUSSION

Path analysis is an exploratory technique that can examine the consequences of particular postulated models (Mitchell-Olds, 1987). It may elucidate complex interactions that would be obscured by multiple regression methods. Path analysis is therefore a useful tool in generating causal hypotheses on ecosystem relations, but it can only indicate whether a particular model is consistent with the data at hand (Mitchell-Olds, 1987). Path analysis can be considered as an

extension of multiple regression analysis (Sokal & Rohlf, 1981). The method allows the investigator to construct possible causal paths with a number of independent and dependent variables. Direct as well as indirect effects can be estimated (Schemske and Horvitz, 1988).

The path diagram of figure 3 suggests that nematode numbers are directly influenced by the C:N ratio of the seaweed detritus, the relative height of the site and the salinity and indirectly by the composition of the sediment and the water-content.

Table 4: Observed and calculated correlation coefficients between decomposition rate and independent variables.

	r_{obs}	r_{calc}
Relative height (cm)	-0.32	-
Coarse sediment fraction (% of DW)	-0.41	-0.39
Fine sediment fraction (% of DW)	-0.18	-0.25
Chloride content ($^{o}/_{oo}$ in Water)	-0.07	-
Water content (% of wet weight)	0.49	0.49

Table 5: Multiple regression models for model II used to construct the path diagram (see figure 4)

Dec. rate = -22.296 + 0.898*Water content - 0.853*Coarse sed. fraction $R^2 = 0.30$

Water content = 90.570 - 0.454*Fine sed. fraction - 0.471*Coarse sed. fraction $R^2 = 0.48$

The model indicates that increasing nitrogen content relative to carbon content (C:N ratio) allows larger nematode populations. This result is in agreement with the findings of Findlay (1982). This author found a positive correlation between the quantity of nitrogen of a substrate and the carrying capacity for populations of the nematode *Diplolaimella chitwoodi*. We found lower C:N ratios on sites in the regions between Shag Point and the Penguin Rookery and near the Penguin Rookery. In these regions many penguins were present and the excrements of these birds may enrich the seaweed deposits with nutrients allowing enhanced population growth of nematodes. On the other hand the seaweed on these sites were probably present for a longer period. A decreasing C:N ratio with progressing decomposition is often found (Valiela, 1985; Rieper-Kirchner, 1989).

The height of the location relative to the high tide level probably is indicative for the time a seaweed package is present on the beach. Presumably the higher a seaweed deposit is located the longer it is present on the beach. After deposition on the beach, nematodes colonize the seaweed wrack and rapid population growth follows (Inglis, 1989), which may explain the positive correlation between the relative height and nematode number.

The salinity is the third direct factor that influenced the nematode number. A linear decrease of nematode number with salinity was observed. We hypothesize that the decrease of nematode number is not a direct consequence of higher salinities but can be ascribed to the different influences of melt water and tidal water on the nematode populations. Both melt water and tidal water are largely responsible for the water content and the salinity of the seaweed deposit.

In the coastal region we studied, we can distinguish three different situations with different influences of melt water and tidal water:

- The first situation occurs in sites where seaweed deposits are regularly flooded by the tide, as is found in the Point Thomas region. The salinity of the moisture in these deposits will be equal to seawater and the water content will be high, due to regularly wetting by tides. The surf will strongly perturb the seaweed deposits. The water may carry away small fragments, with adhering nematodes resulting in lower nematode numbers.

- In the second situation the influence of tidal inundations and melt water run-off is low. This occurs in the region between Shag Point and the Penguin rookeries. The salinity in the seaweed debris is mainly influenced by desiccation and precipitation. As desiccation is relatively limited a slightly lower salinity than seawater caused by precipitation is expected. The water content, however, is lower. The nematode population growth is not inhibited by desiccation nor by running water, and in this environment nematode populations may increase rapidly.
- In the third situation seaweed deposits are present in melt water run-off streams. This occurs near Ecology Glacier and in the Penguin Rookery region. The salinity is lowered by the melt water and the water content will be high, due to continuous wetting. The continuous fresh water stream over the seaweed deposits does not disturb the seaweed in a way tides do. Slow running water apparently does allow nematode population growth and may even stimulate it. Some nematodes, however, will be carried away by the water.

Following the above line of reasoning, we thus interpret the correlations between salinity, water content and nematode number found by the path analysis as follows: the negative correlation between salinity and nematode number resulted from the limited population growth in situations with high tidal influences; the low correlation between water content and nematode number presumably is found because both in situations with tidal water and in those with melt water influences the seaweed deposits have a high water content, but only in situations with melt water run-off the nematode numbers can be high; the negative correlation between water content and salinity we ascribe to the effect of the melt water input.

Mean decomposition rate was low in the region between Shag Point and Rakusa Point but in the other regions the decomposition rates were in the range found in literature. Zielinsky (1981) found in the same area that in the summer season about 55 % of initial dry weight was lost in 20 days. Decomposition rates of seaweed on sandy beaches in more temperate region are very similar. Inglis (1989) found that 36-59 % of the original dry weight remained after 18 days of exposure on a sandy beach in New Zealand. On a beach in South Africa weight

losses between 60 and 80 % in 20 days were found (Griffith & Stenton-Dozey, 1981). In the regions near Point Thomas, near the Penguin Rookeries and near the Ecology Glacier the mean decomposition rates were slightly lower than the values found by Zielinski (1981) and within the range found in New Zealand (Inglis, 1989).

The model (fig 4), resulting from the path analysis with decomposition rate as dependent variable, suggests lower decomposition rates in drier situations. The sediment composition influences decomposition rates both directly and indirectly. The effect of water content on decomposition rates may have two different causes. In the first place the bacterial respiration may decrease when decomposing material dries out (see e.g. Newell et al., 1985). Secondly, as was mentioned above, high water contents presumably point to either high tidal influences or melt water flows. Particularly the recurrent surf during tidal inundations is expected to carry away small fragments from the decomposing seaweed into the sea, resulting in higher weight losses.

In conclusion this study indicate that, apart from the time a seaweed deposit is present on the beach and the C:N ratio of the seaweed, tidal influences and melt water run-off are important factors determining nematode numbers in seaweed deposits on Antarctic beaches. Tidal inundations and melt water flows probably also influenced weight losses of seaweed wrack.

CHAPTER FOUR

Stimulation of decomposition of *Spartina anglica* leaves by the bacterivorous marine nematode *Diplolaimelloides bruciei* (Monhysteridae)

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ABSTRACT

The bacterivorous nematode *Diplolaimelloides bruciei* was found in association with above-ground decaying plant parts of the halophyte *Spartina anglica*. The effect of this nematode on decomposition of *S. anglica* leaves was investigated in laboratory experiments. CO_2 production, losses of dry weight, carbon and nitrogen of the decaying leaves were examined. The influence of leaf age was investigated in these experiments.

In the presence of nematodes, CO_2 production of green, decaying leaves increased by 20 - 25 %. Losses of dry weight, carbon and nitrogen during decomposition increased with at least 30 %. On yellow, more senescent, leaves no effect on CO_2 production was found, but losses of dry weight, carbon and nitrogen tended to be higher in the presence of nematodes.

In the presence of nematodes more carbon was lost from the leaves than could be explained by CO_2 production alone. Budget calculations indicate that the difference was mainly due to incorporation of carbon in nematode biomass.

The minimal nematode population density at which the nematode have any measurable stimulatory effect was estimated at 4000 individuals per gram of dry leaves. Field population densities are often of the same order of magnitude.

The results showed clearly that *D. bruciei* enhanced the decomposition rate of *S. anglica*-leaves. The extent depends on leaf condition and the population density of this nematode.

INTRODUCTION

Spartina anglica Hubbard is a dominant halophyte of salt marshes of the South-Western part of the Netherlands. The plant frequently forms dense monospecific stands in the lower parts of salt marshes.

Senescent and dead leaves may remain attached to the standing stems for a prolonged period (Groenendijk, 1984; Buth & Voesenek, 1988). More than 90 % of the dead leaves of *Spartina alterniflora* were still attached to the plant

after 147 days (Newell et al., 1989). Leaf decomposition starts on the still standing plants.

Large numbers of the nematode *Diplolaimelloides bruciei* Hopper are present on the surface of decaying *S. anglica* leaves (Bouwman et al., 1984; Warwick, 1981). *D. bruciei* is generally considered to be a bacterivorous nematode (Nicholas, 1984; Romeyn and Bouwman, 1983), and does not feed on the *Spartina*-derived detritus itself. The food source of this nematode is the bacterial population living on the leaves. The relationship between *D. bruciei* and *S. anglica* seems to be very specific as the nematode has not yet been isolated from other substrata. (Bouwman et. al. 1984).

The effect of bacterivorous nematodes on decomposition has been mainly studied in terrestrial ecosystems. Generally carbon and nitrogen mineralization rates are increased in the presence of nematodes. (e.g. Anderson et al., 1981; Trofymov et al., 1983; Ingham et al., 1985). Only fragmentary information exists on the influence of nematodes on decomposition processes in marine environments. (Findlay & Tenore, 1982; Rieper-Kirchner, 1989; Tietjen & Alongi, 1990).

In the present study we investigated the effect of *D. bruciei* on decomposition rates of *S. anglica* leaves. *D. bruciei* may enhance decomposition rates by stimulating the bacterial population living on the surface of *S. anglica* leaves.

Decomposition rates of *S. anglica*-leaves in presence and absence of nematodes were examined in laboratory experiments. Decomposition can be measured by CO_2 production or by determining the losses of dry weight. However, if part of the carbon is incorporated in other elements of the system, the rates of dry weight losses and the CO_2 production will not match. Therefore both CO_2 production and losses of dry weight were determined.

The activity of the microbial community on *S. anglica* leaves changes during decay in the canopy (Buth & Voesenek, 1988), probably as a result of the changing resource quality of the decaying leaves. A difference in resource quality may alter the effect of nematodes on decomposition (Findlay & Tenore, 1982). Therefore, we included the influence of leaf age in this study.

MATERIAL AND METHODS

Nematode culture

Specimens of *D. bruciei* were obtained from *S. anglica* leaves collected from the salt marsh near Stroodorpepolder in the Oosterschelde, a tidal inlet of the North Sea. Fragments of decaying leaves of *S. anglica* were placed in extraction petri dishes (10 cm. diameter) with a 2 mm. layer of bacto-agar (DIFCO, 0.8 %; in autoclaved seawater with a salinity of 26 $^{o}/_{oo}$; Vranken et al., 1981). The nematodes dispersed from the leaf material into the agar; using a dissecting microscope adult *D. bruciei* specimens were picked up with a fine needle and were transferred to petri dishes with bacto-agar. After about one hour they subsequently were transferred to culture dishes. In these transfer steps, contaminating ciliates and other protozoans were removed. Culture dishes (∅ 3.5 cm) contained 3 ml autoclaved Oosterschelde seawater with 0.5 % bacto-agar, 1 % Vlasblom medium and 0.5 % of a 15 g/l solution of $Na_2SiO_3.9H_2O$ (Vranken et al., 1988). The dishes were inoculated with 10 - 20 adult *D. bruciei* and a droplet of a bacterial inoculum obtained by filtering seawater over a 1.2 µm sterile micropore filter.

The nematodes rapidly reproduced in the culture dishes: after 6 weeks the population generally increased to several thousands. Cultures were renewed every 6 weeks by transferring 100 µl medium from the old culture dishes to a petri dish with fresh medium.

Experiments

Petri dishes (∅ 3.5 cm) were filled with 2.5 ml bacto-agar (0.8 % in autoclaved seawater). TRIS buffer was added to a final concentration of 5 mM to maintain a pH of 7 - 8 during the experiments.

In January 1990, leaves of *S. anglica* were collected on the Stroodorpepolder salt marsh and cleaned with wetted cottonwool and a small brush. The leaves were surface sterilized for 45 minutes by immersion in 96 % alcohol, and subsequently rinsed with autoclaved seawater.

In the first experiment green leaves of similar length (10 cm) were used. Each leaf was cut into four pieces; these pieces were weighed and divided over two petri dishes, two randomly selected pieces were placed in each dish. One petri dish served as a control, the other received nematodes. The experiment was done with four of such sets of two dishes arranged in a randomized block design. An additional batch of leaves was used for determining the relation between wet and dry weight, and the initial content of carbon and nitrogen.

In a second, similar, experiment both green and yellow (more senescent) leaves were used. The leaves were cut into pieces of about 2 cm length and weighed. Dishes received two randomly selected pieces of leaf of similar age. Per treatment four replicate dishes were prepared.

In both experiments a droplet of a natural bacterial inoculum was added to each petri dish. The inoculum was obtained by rinsing decaying *S. anglica* leaf material with autoclaved seawater and filtering the suspension over a 1.2 µm. micropore filter. We assumed that the *S. anglica* leaves in the petri dishes were the only substrate available for the bacteria.

Subsequently, adult specimens of *D. bruciei* were added. In order to minimize transfer of bacteria from the agar of the culture dishes to the experimental dishes, nematodes were first brought from the culture dishes into dishes with bacteria-free bacto agar for 1 hour before transferring them to the experimental dishes.

In the first experiment 10 females and 3 males per dish were inoculated. In the second experiment 6 females and 2 males were used. Control dishes received no nematodes. The dishes were placed in the dark, in an incubator with water saturate air, at 20 °C.

CO_2 production was measured regularly during the course of the experiment using gaschromatography (Mitchell, 1973; Abram & Mitchell, 1980). The petri dishes were incubated in an air-tight chamber (20 ml) for 1 to 4 hours (dependent on the CO_2 production in the dish). CO_2-concentration in the incubation chambers was measured before and after incubation by injecting air samples on a Packard 427 gaschromatograph equipped with a stainless steel column (1/8 inch * 2 m) packed with Haysep Q (60 - 80 mesh) and connected with a Chrompack micro thermal conductivity detector. The injector and column temperatures were 200°C and 50°C, respectively, and detector and filament

temperatures were 120°C and 200°C, respectively. The carrier gas was Helium (flow rate 20 ml min^{-1}).

Cumulative CO_2 production was calculated from CO_2 production measured on subsequent sampling days by linear interpolation and subsequent integration.

At the end of the experimental period the pieces of leaf were picked up carefully and nematodes were rinsed off with water. These nematodes and the nematodes which remained in the dishes were fixed with warm formalin (4 %) for later counting. The leaf fragments were dried and weighed and the carbon and nitrogen content of the pooled fragments of the replicate dishes were determined. All nitrogen and carbon analyses were carried out with a Carlo Erba Nitrogen/Carbon analyzer type NA 1500.

The nematodes were counted under a dissecting microscope. Biovolume was estimated by measuring length and width of 100 randomly chosen nematodes and using the Andrassy formula (Feller & Warwick, 1988). We assumed that 1 nl of nematodes equals 1.13 µg. (Heip et al., 1985).

Analysis of variance was used for evaluating differences in CO_2 production per hour, cumulative CO_2 production, losses of dry weight, carbon and nitrogen.

RESULTS

CO_2 production

In the first experiment the cumulative CO_2 production (fig. 1) was significantly higher in treatments with nematodes from day 7 onwards ($P < 0.05$). At the end of the experiment, the cumulative CO_2 production in dishes with nematodes exceeded the production of the control by 25 %.

CO_2 production per hour in both nematode treated and untreated dishes increased rapidly in the first two days and declined slowly to a more or less stable level after about 20 days (fig. 2). The CO_2 production per hour tended to be higher in the presence of nematodes, but was only significant at the end of the sampling period, i.e., after 21, 24 and 32 days ($P < 0.05$).

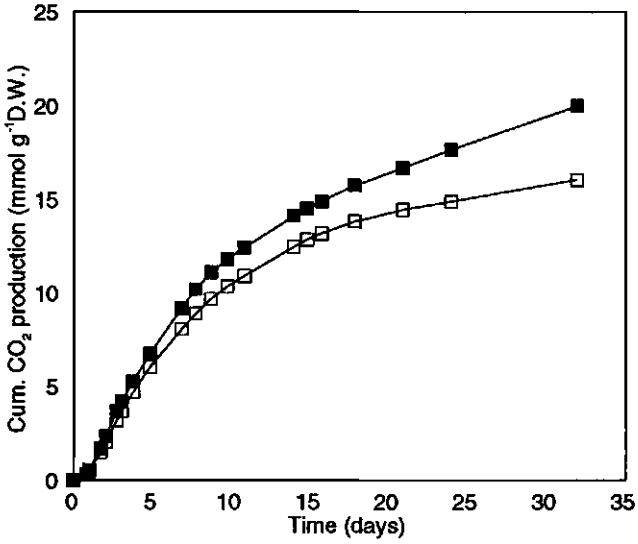


Figure 1: Cumulative CO_2 production in dishes containing fragments of green *Spartina anglica* leaves, with and without nematodes. Each line is the mean of four replicates. (■ = with nematodes, □ = without nematodes)

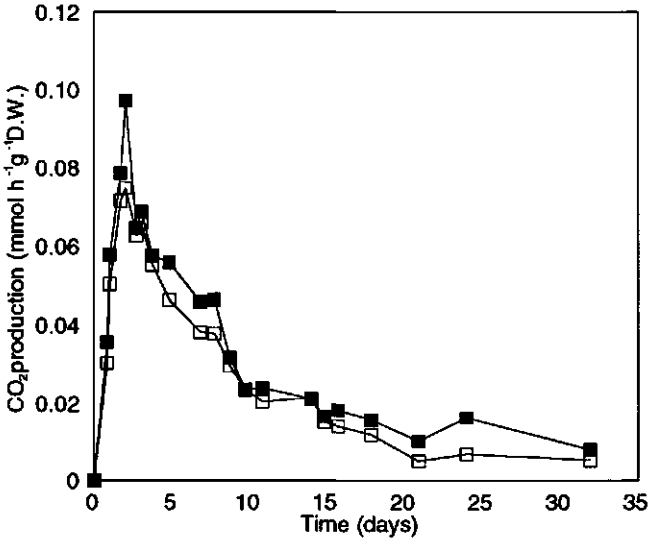


Figure 2: CO_2 production in dishes containing fragments of green *Spartina anglica* leaves, with and without nematodes. Each line is the mean of four replicates. (■ = with nematodes, □ = without nematodes)

In the second experiment patterns of cumulative CO_2 production (fig. 3) and CO_2 production per hour (fig. 4) were similar to those in the first experiment. Cumulative CO_2 production during the course of the experiment in dishes with green leaves was about 3 times higher than in dishes with yellow leaves ($P < 0.001$). In dishes with green leaves cumulative CO_2 production was conspicuously higher in treatments with nematodes. However, due to the relatively large variation and the absence of data between day 12 and 30 these differences were not statistically significant.

CO_2 production per hour in dishes with green leaves was higher than in dishes with yellow leaves. No significant differences between treatments with nematodes and control treatments were found. Only on the last sampling day CO_2 production per hour was significantly higher in treatments with nematodes ($P < 0.01$). A significant interaction between age of the leaf material and presence or absence of nematodes was found ($P < 0.01$). An a posteriori multiple comparison test showed that only in treatments with green leaves CO_2 production per hour was higher in the presence of nematodes.

Weight losses and losses of carbon and nitrogen

Table 1 shows initial carbon and nitrogen content of the leaves. The C:N ratios of green leaves in both experiments were approximately similar. Yellow leaves had a conspicuously higher C:N ratio. The values were similar to those reported by Buth & de Wolf (1985) for green and yellow leaves.

Table 1: Initial Carbon and Nitrogen content expressed as percentages of total dry weight and C:N ratios of green and yellow *Spartina*- leaves.

	Experiment 1	Experiment 2	
	Green	Green	Yellow
percentage C	41.0	38.0	35.8
percentage N	2.66	2.04	0.89
C:N ratio	15.41	18.65	40.28

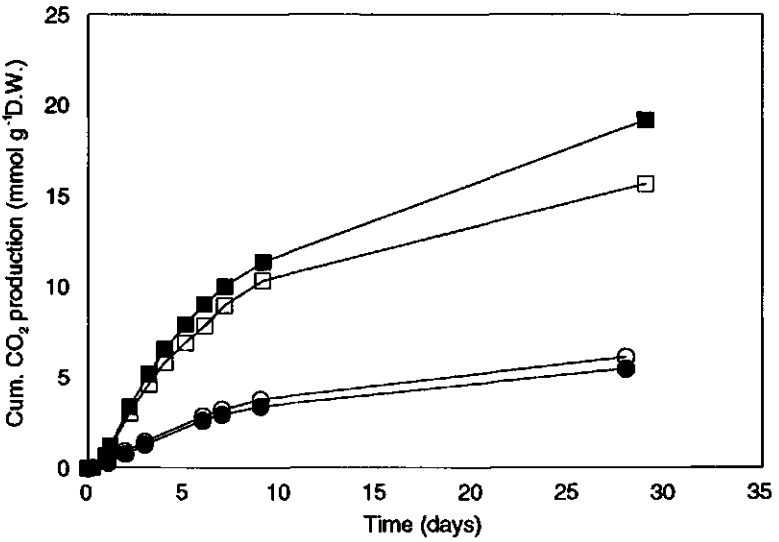
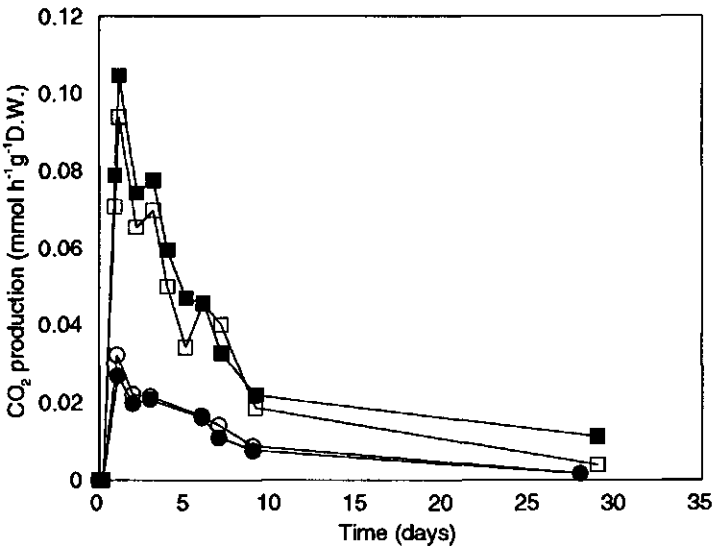


Figure 3: Cumulative CO_2 production in dishes containing fragments of green and yellow *S. anglica* leaves, with and without nematodes. Each line is the mean of four replicates. (■ = green leaves with nematodes, □ = green leaves without nematodes, ● = yellow leaves with nematodes, ○ = yellow leaves without nematodes)



CO_2 production in dishes containing fragments of green and yellow S. anglica leaves, with and without nematodes. Each line is the mean of four replicates. (■ = green leaves with nematodes, □ = green leaves without nematodes, ● = yellow leaves with nematodes, ○ = yellow leaves without nematodes)

Table 2: Losses of weight, carbon and nitrogen of green and yellow *Spartina*-leaves, expressed as percentages of the initial amount.

		Experiment 1	Experiment 2	
		Green	Green	Yellow
Weight	with nem.	63.0	65.4	31.2
	without nem.	47.0	51.6	19.2
Carbon	with nem.	64.3	62.7	20.0
	without nem.	49.2	48.7	7.5
Nitrogen	with nem.	78.7	65.0	37.3
	without nem.	54.3	52.3	22.4

Table 2 shows the losses of dry weight, carbon and nitrogen as percentages of initial values. The differences of initial weights between treatments were minor and statistically insignificant. The initial dry weight of the leaf fragments was approximately 15 mg on average per dish.

In the first experiment losses of leaf dry weight were significantly greater ($P < 0.01$) in the presence of nematodes. Losses of carbon and nitrogen similarly were significantly higher in the presence of nematodes ($P < 0.01$ and $P < 0.05$, respectively).

In the second experiment, the presence of nematodes significantly increased total weight loss, total loss of carbon and total loss of nitrogen as well ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively). Losses of weight, carbon and nitrogen were significantly larger in treatments with green leaves than in treatments with yellow leaves ($p < 0.001$) (Table 2).

In Table III the increased losses of dry weight, carbon and nitrogen and the increased CO_2 production in the presence of nematodes, expressed as relative changes compared to control values are summarized. Comparatively high values for the losses of weight, carbon and nitrogen in treatments with yellow leaves were found.

Table 3: Relative changes in losses of dry weight, carbon and nitrogen and released CO_2 in treatments with nematodes compared to control treatments (=100 %)

	Experiment 1	Experiment 2	
	Green	Green	Yellow
Loss of weight	34	27	62
Loss of Carbon	31	29	167
Loss of Nitrogen	45	24	66
released CO2	25	23 *	-12 *

* = statistically non-significant, other values are significant ($P < 0.05$).

The relative changes in losses of weight, carbon and nitrogen in treatments with green leaves were consistently larger than the relative change in CO_2 production.

Nematode counts and biomass estimates

At the end of the first experiment the average number of nematodes per petri dish had increased to 33,000 individuals, corresponding with a density of $2.2*10^6$ individuals per gram of dry *S. anglica* leaves. 92 % of the nematodes were juveniles, 6 % females and 2 % males. Calculated mean biomass per dish was 1.65 mg., so that total biomass had increased a 250-fold in 35 days.
In the second experiment nematode numbers increased to, on average, 17,000 individuals per dish ($1.13*10^6$ per gram of dry leaves) in treatments with green leaves and to 11,000 individuals ($0.73*10^6$ per gram of dry leaves) in treatments with yellow leaves. Percentage of juveniles, females and males on treatments with green leaves were 91 %, 7 % and 2 %, respectively. The calculated nematode biomass per dish was, on average, 0.87 mg.. On yellow leaves 96 % of the nematodes were juveniles, 3 % were females and 1 % males. The calculated biomass was, on average, 0.39 mg.. Total biomass increased a 210-

fold in treatments with green leaves and a 100-fold in treatments with yellow leaves in 30 days.

DISCUSSION

Using Gaschromatography CO_2 production and O_2 consumption could be measured simultaneously. Abram & Mitchell (1980) considered O_2 consumption as a better index of microcosm metabolism. In their microcosms carbon dioxide was partially absorbed in the medium and consequently the CO_2 production was underestimated. In pilot experiments we found that CO_2 production values were consistently about 80 % of the values for O_2 consumption and therefore using either CO_2 production or O_2 consumption give similar patterns of results.

In the presence of the bacterivorous nematode *D. bruciei* the CO_2 production, the loss of dry weight and the losses of carbon and nitrogen of decaying still green *S. anglica*-leaves increased significantly, as is summarized in table 3, indicating that the decomposition rate of *S. anglica*-leaves was enhanced by *D. bruciei*.

Experimental data on the effect of nematodes on decomposition processes in marine environments were first provided by Findlay & Tenore (1982). They found an increased CO_2 production of *Spartina* and *Gracilaria* detritus in the presence of the bacterivorous nematode *Diplolaimella chitwoodi*. Their experiments, however, were short-term and the differences they found were not statistically significant (minimum P value was 0.13). Rieper-Kirchner (1989) found, as a single observation, increased dry weight losses of degrading seaweed detritus in the presence of nematodes. Tietjen & Alongi (1990) found no significant effects of nematodes on nutrient regeneration of mangrove detritus. They stated that the lack of effect was related to the relatively low numbers of nematodes in their experiments. In our experiments the effect of the nematodes was conspicuous and statistically significant.

The effect of the nematodes on CO_2 production increased during the course of the experiments. In the first experiment the cumulative CO_2 production in treatments with nematodes exceeded control treatments with 15 % on day 7 and 25 % at the end of the experiment. This is probably related to the population

growth of the nematodes. A rough minimum estimate of the population density during the course of the experiment can be obtained by assuming exponential growth of the nematode population. Using the initial and the final density the population growth rate **r** can be calculated. The growth rate in the first experiment was 0.22 d^{-1}. This figure is almost equal to the population growth rates found by Warwick (1981) for the same species. Thus the assumption of exponentional growth in this case appears reasonable. Day 7 is the first sampling day on which the cumulative CO_2 production in the treatment with nematodes exceeded, statistically significantly, the control treatment. On that day the population density was estimated to be about 4000 individuals per gram of *S. anglica* dry weight. This figure might be the minimum population density leading to a measurable stimulatory effect. In field situations the population densities are frequently in the same order of magnitude (see chapter II), and effects of this nematode on decomposition processes in the salt marsh therefore can be expected.

The increased weight loss in treatments with nematodes relative to control treatments was higher than the relative change of CO_2 production (see table 3). This discrepancy to a large extent can be explained by the incorporation of carbon originating from *Spartina* detritus in nematode biomass. This is demonstrated by calculating a carbon budget.

Table 4: Carbon budget of decomposed green *Spartina* leaves after 35 days of incubation, with and without nematodes, expressed in percentages of initial amount.

	With	Without
Initial	100	100
Remaining	36	51
Mineralized (CO_2)	56	44
Biomass nematodes	3	-
Unexplained	5	5

Table 4 shows this for the data of the first experiment. For calculation of carbon equivalents in nematode biomass we assumed that the carbon content of

nematodes is 10.6 % of their wet weight (Sikora et al., 1977; Heip et al., 1985). Furthermore we assumed that the biomass of nematodes and bacteria at the beginning of the experiment was neglectable compared with the carbon input from the leaf material. In treatments with nematodes the explained loss of carbon is 59 %. This figure is calculated from table 4 by adding the amount of carbon released through CO_2 (56 %) and the amount incorporated in nematode biomass (3 %). The explained loss of carbon (CO_2 released) in control treatments is 44 %. The relative change of the explained loss of carbon in treatments with nematodes is then 34 % compared with control treatments. This figure is slightly higher than the loss of carbon given in table 3 (31 %).

In the second experiment the CO_2 production increased in the presence of nematodes in treatments with green leaves but not in treatments with yellow leaves. The increases of the losses of dry weight, carbon and nitrogen were relatively large in treatments with yellow leaves, although the accuracy of these figures was negatively influenced by the relatively small weight changes found in the control treatments with yellow leaves. The different results found with green and yellow leaves may indicate that the effect of *D. bruciei* on decomposition of *S. anglica* leaves varies with the condition of the leaf material. This conclusion is in agreement with earlier observations of Findlay & Tenore (1982), who found that the closely related nematode species *Diplolaimella chitwoodi* increased the rate of carbon mineralization of *S. alterniflora* detritus to a lesser degree than *Gracilaria sp.*, which has a higher nutritional quality (Tenore, 1981).

Our observations suggest that the effect of *D. bruciei* on the decomposition rate of *S. anglica* leaves is not straightforward, but depends on different factors. The present results indicate that leaf condition and nematode population density are important factors modulating the effect of *D. bruciei*.

The increased rate of decomposition is due to the stimulation of the bacterial population by the nematodes as they do not feed on the *Spartina*-derived detritus itself (Bouwman et al., 1984).

Several possible mechanisms of nematode stimulation of bacteria have been suggested in the literature. Grazing by nematodes may stimulate a bacterial population by maintaining bacterial growth at a higher rate (Gerlach, 1978).

However, Herman & Vranken (1988) calculated that the grazing rates of nematodes are not high enough to stimulate bacterial growth in this way. Nematodes may speed up nutrient cycling so that the availability of nutrients increases (Ingham et al., 1985). Bioturbation by nematodes may facilitate gas exchange and increase the oxygen supply for bacterial populations. Mucus secreted by nematodes may attract and sustain bacterial populations (Riemann & Schrage, 1978).

Our findings do not yield evidence with regard to the specific mechanism of stimulation of the bacterial population. But whatever the mechanism may be, the present results clearly show that *D. bruciei* accelerates the decomposition of *S. anglica* leaves.

CHAPTER FIVE

Experimental evidence for the role of bioturbation by the marine nematode *Diplolaimella dievengatensis* in stimulating the mineralization of *Spartina anglica* detritus.

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ABSTRACT

The effect of the bacterivorous nematode *Diplolaimella dievengatensis* on the diffusion of oxygen into sediment and the CO_2 production of *Spartina anglica* detritus was examined in a laboratory experiment. Diffusion coefficients were calculated from measurements of both O_2 consumption, using gas chromatography, and O_2 micro-gradients, using micro-electrodes. After a transient state of about 10 days the diffusion and consumption of oxygen stabilized and approached steady state. In treatments with nematodes O_2 consumption and CO_2 production were 74 % higher than in controls. In treatments with nematodes the apparent diffusion coefficient of oxygen was 40 to 70 % higher than the molecular diffusion coefficient due to nematode activity. Since the increases of CO_2 production and of the diffusion of oxygen in the presence of nematodes were of the same magnitude, we conclude that the enhanced turnover time of *Spartina* detritus was largely dependent on the bioturbation activity of the nematodes.

INTRODUCTION

Bacterivorous nematodes of the family Monhysteridae accelerate decomposition processes in marine sediments. *Diplolaimella chitwoodi* increased the detrital carbon mineralization of *Gracilaria* and *Spartina* detritus (Findlay and Tenore, 1982). In the presence of *Monhystera disjuncta* increased weight losses of seaweed detritus were found by Rieper-Kirchner (1989). In a previous study we found an increased mineralization of organic carbon and increased weight losses of *Spartina anglica* leaves in the presence of *Diplolaimelloides bruciei* (Alkemade et al., 1992). The mechanism by which nematodes accelerate decomposition processes is still unknown. Three possible mechanisms have been suggested. Firstly, removal of senescent bacterial cells by grazers might stimulate bacterial growth by keeping the bacterial population active (Abram & Mitchell, 1980). Secondly, Riemann & Schrage (1978) suggested that mucus produced by nematodes provides a rich food source for bacteria resulting in increased bacterial growth. Bioturbation has been suggested as a third mechanism by which nematodes stimulate bacterial activity (Abram and Mitchell, 1980; Herman and Vranken, 1988). Bioturbation is the

process resulting from the mixing activities of benthic organisms (Berner, 1980). The mixing activities enhance the diffusion of oxygen (Hofman et al., 1991) and would thereby stimulate aerobic bacterial activity.

In this study we investigated the effects of the bacterivorous nematode *Diplolaimella dievengatensis* on the diffusion of O_2 and the mineralization of organic carbon in an artificial sediment enriched with *Spartina anglica*-detritus. *D. dievengatensis* is frequently found in sediments of salt marshes with *S. anglica*-vegetation in the South-Western part of the Netherlands. Bioturbation was estimated from the diffusion coefficient of oxygen. An enhanced diffusion coefficient relative to molecular diffusion is a measure for bioturbation (Berner, 1980). The diffusion coefficient was determined by combined measurements of O_2 consumption rates and O_2 micro-gradients. Mineralization of organic carbon (measured as CO_2 production) was determined simultaneously.

MATERIAL AND METHODS

Nematode culture

Specimens of *D. dievengatensis* were extracted from the sediment as described by Vranken et al. (1981). Culture dishes containing bacto-agar, Vlasblom-medium and $Na_2SiO_3.9H_2O$ (Vranken et al., 1988) were inoculated with 10 - 20 adult specimens of *D. dievengatensis* and a droplet of filtered (1.2 µm) seawater. Before inoculating the nematodes to the culture dishes an extra transfer step was performed in order to avoid contamination by ciliates and other protozoa (Alkemade et al. 1992).

Experimental design

Green *S. anglica* leaves were collected on the Stroodorpepolder salt marsh in the Oosterschelde, a tidal inlet of the Southern North Sea. Leaves were dried at 50°C for 24 hr., ground and sieved. Sand was cleaned with HCl and H_2O_2 in order to remove organic matter, and subsequently sieved. Leaf particles and sand grains which passed a 90 µm mesh sieve and that were retained on a 45 µm mesh sieve

were used in the experiment. The leaf particles were mixed with sand and 8 petri dishes (∅ 3.5 cm) were filled with a 6 mm thick layer of this mixture. The initial organic carbon content of the mixture was 0.36 %, a value found in many natural sediments. Autoclaved seawater from the Oosterschelde (salinity 26 ‰) was added. A very thin water film was present on the sediment, indicating water-saturated conditions.

The dishes were inoculated with a natural bacterial inoculum, obtained by rinsing decaying *S. anglica* leaves in autoclaved seawater and subsequently filtering the water over a 1.2 µm micropore filter. Each dish received 100 µl of the inoculum. Finally, 50 to 100 nematodes were added to 4 dishes; the others served as control treatments. The dishes were placed in the dark in an incubator at 20°C in water-saturated air.

After 22 days, at the end of the experiment, nematodes were fixed in warm formalin, extracted from the sediment and counted under a dissecting microscope. The nematode biomass was estimated by the volumetric method of Andrassy (Feller & Warwick, 1988).

CO_2 production and O_2 consumption measurements

CO_2 production and O_2 consumption were measured regularly during the course of the experiment using gas chromatography (Mitchell, 1973; Abram and Mitchell, 1980). The petri dishes were incubated at 20°C, in the dark, in an air-tight chamber (20 ml) for 1 to 4 hours (dependent on the CO_2 production in the dish). CO_2 and O_2 concentration in the incubation chambers were measured before and after incubation by injecting air samples on a Packard 427 gas chromatograph (details in: Alkemade et al., 1992). The CO_2 and O_2 flux are expressed as the CO_2 production and O_2 consumption per m^2 per hour. Cumulative CO_2 production and O_2 consumption were calculated from CO_2 and O_2 fluxes measured on subsequent sampling days by linear interpolation and subsequent integration.

Oxygen micro-profiles were measured with oxygen micro-electrodes as described by Revsbech and Jørgensen (1983) and de Jong et al. (submitted). The oxygen micro-profile is graphically represented by plotting the oxygen concentration, measured at one sampling time, against depth. The oxygen micro-gradient is referred to as the slope of the micro-profile calculated by linear regression. The

micro-electrodes were constructed at our institute. The theory of using micro-electrodes in mineralization processes is described in Hofman et al. (1991).

Calculation of the apparent sediment diffusion coefficient.

The oxygen flux into the sediment can be described by Fick's first law of diffusion (Berner, 1980):

$$J_{O_2} = -\phi D_a \left(\frac{dO_2}{dx}\right)_{x=0} \qquad (1)$$

where J_{O2} = flux of oxygen (mmol O_2 m^{-2} h^{-1}); ϕ= mean porosity for oxic sediment layer, defined as the volume of water relative to the volume of total sediment; D_a = apparent diffusion coefficient (m^2 h^{-1}) and dO_2/dx = oxygen micro-gradient over depth interval x (mmol O_2 m^{-4}).
The apparent diffusion coefficient is calculated by rewriting (1) in the form:

$$D_a = -\frac{J_{O_2}}{\phi \left(\frac{dO_2}{dx}\right)_{x=0}} \qquad (2)$$

The flux of oxygen (J_{O2}) was calculated from the rate of change of oxygen concentration measured in incubation chambers by gas-chromatography. Exact values of D_a could not be calculated, because the micro-gradient depends on the depth interval from which it is derived. This depth dependence may be due to porosity changes with depth (e.g. Booij et al., 1991; Archer et al., 1989). In order to determine a range for the diffusion coefficient, we estimated the oxygen micro-gradient using 4 different depths intervals. The series of oxygen micro-gradients calculated per profile together with the corresponding independent estimates of the oxygen fluxes were used for calculation of the apparent diffusion coefficients. Estimates, obtained by linear interpolation, of the oxygen fluxes were used, because fluxes and micro-profiles could not be measured at the same time. In the absence of bioturbation the apparent diffusion coefficient equals the bulk sediment diffusion coefficient (D_s); in the presence of bioturbation the apparent diffusion coefficient is higher.
The bulk sediment diffusion coefficient is calculated by correcting the free solution

diffusion coefficient (D_0) for mean porosity of the sediment (Hofman et al., 1991; Broecker & Peng, 1974). Since we found a mean porosity of about 0.50 the bulk sediment diffusion coefficient is calculated from: $D_s = D_0 * \phi$, according to Ullman and Aller (1982).

Statistical analysis

Measurements of O_2 consumption, CO_2 production and O_2 micro-gradients were initially carried out daily in order to detect the beginning of a stabilized situation. In a stabilized situation no major changes occur between succesive sampling days. The diffusion and the consumption of oxygen then approach a steady state. Time lapses between sampling days were longer in the steady state period. Multivariate analysis of variance with a repeated measures design was used to evaluate the differences found between treatments with and without nematodes during the steady state period. SYSTAT (Wilkinson, 1990) was used for statistical calculations.

RESULTS

CO_2 production and O_2 consumption

At the beginning of the experiment the O_2 consumption, CO_2 production and O_2 micro-gradients changed rapidly from day to day (Figures 1 and 3). During this "transient state" the CO_2 production per hour was high and decreased gradually to a more or less stable level after about 5 days. No major changes of gas fluxes occurred after the first 5 days, but the micro-gradients and the maximal penetration depth of oxygen remained variable until after about 10 days (see below). The period from 10 days onwards to the end of the experiment is considered to represent a steady state.

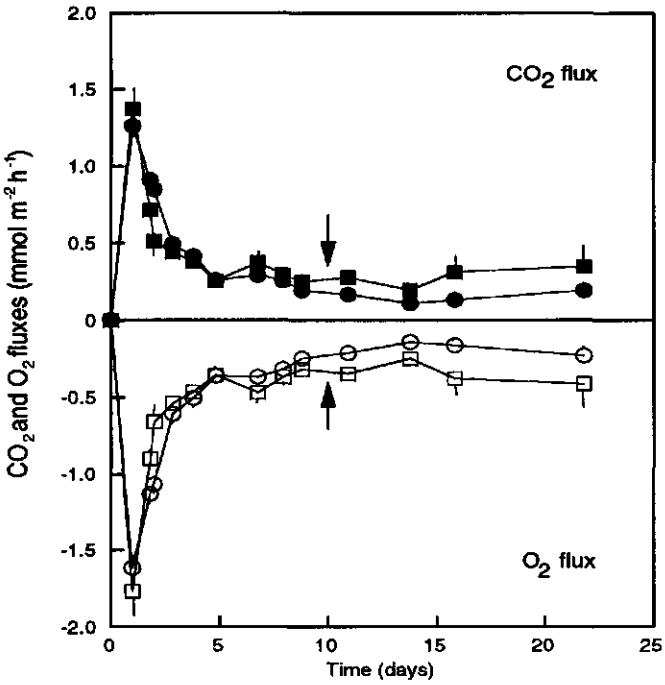

Figure 1: CO_2- and O_2-fluxes in the sediment during the course of the experiment. (■ CO_2-flux with nematodes, ● CO_2-flux without nematodes, □ O_2-flux with nematodes, ○ O_2-flux without nematodes). All point are mean values of 4 replicates. Vertical bars indicate 1 standard error. Arrow heads indicate the beginning of the steady state period.

O_2 consumption per hour was an almost identical reflection of the CO_2 production pattern (Figure 1). The respiratory quotient (= $-dCO_2/dO_2$) ranged from 0.77 to 0.84 (0.80 on average), which indicates that carbon was accumulated in bacterial and nematode biomass during the course of the experiment. In the presence of nematodes more CO_2 was produced and more O_2 was consumed per hour during the steady state. This resulted in a 26 % higher cumulative CO_2-production in treatments with nematodes at the end of the experiment (figure 2). The cumulative CO_2-production was significantly higher ($P < 0.05$, table 1) during the steady state period.

Table 1: Multivariate repeated measures analysis: Univariate test statistics of (A) cumulative CO_2-production, (B) Apparent oxygen diffusion coefficients calculated from the depth interval 0.05-1.0 mm and (C) oxygen penetration depth.

	Variable	SS	DF	MS	F	P
(A)						
	Between subjects					
	Nematodes	2661	1	2661	7.36	0.035
	Error	2169	6	362		
	Within subjects					
	Time	20380	2	10190	39.41	0.001
	Time*Nem.	2040	2	1020	3.95	0.048
	Error	3103	12	259		
(B)						
	Between subjects					
	Nematodes	140	1	140	11.00	0.016
	Error	77	6	13		
	Within subjects					
	Time	3.07	2	1.53	0.31	n.s.
	Time*Nem.	19.91	2	9.95	2.01	n.s.
	Error	59.29	12	4.94		
(C)						
	Between subjects					
	Nematodes	0.74	1	0.74	8.83	0.025
	Error	0.50	6	0.08		
	Within subjects					
	Time	0.11	2	0.06	1.79	n.s.
	Time*Nem.	0.02	2	0.01	0.36	n.s.
	Error	0.38	12	0.03		

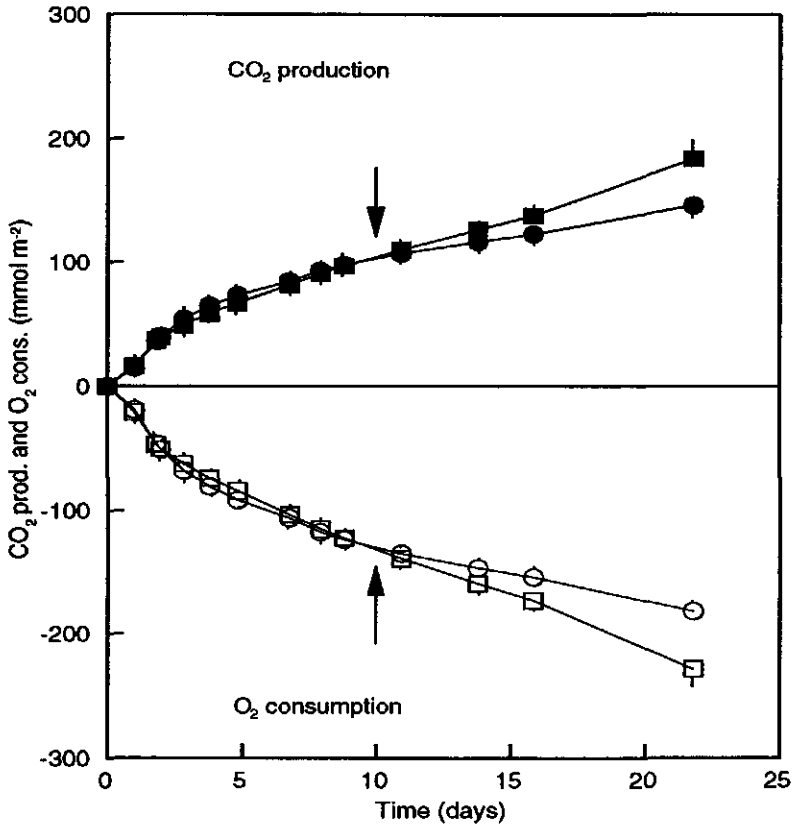

Figure 2: Cumulative CO_2-production and O_2-consumption in the sediment during the course of the experiment. (■ CO_2-production with nematodes, ● CO_2-production without nematodes, □ O_2-consumption with nematodes, ○ O_2-consumption without nematodes). All point are mean values of 4 replicates. Vertical bars indicate 1 standard error. Arrow heads indicate the beginning of the steady state period.

Oxygen micro-profiles

The micro-profiles in sediments measured on day 3, in the transient state period of the experiment, and on day 11, in the steady state period, are shown in figure 3. Profiles with (Fig. 3A) and without nematodes (Fig. 3B) are shown. During the transient state, the oxygen micro-profiles in the sediment were steep and changed

from day to day, corresponding with high O_2-fluxes at the beginning of the experiment (see figure 1). After 10 days micro-profiles were, in general, less steep and no major changes occurred, corresponding with the stabilization of the O_2 fluxes. During the course of the experiment the oxygen was only present in the upper few millimeters. In this upper layer the bacteria apparently were active and consumed all oxygen diffusing into the sediment. In deeper layers no oxygen was present and therefore no aerobic mineralization took place. At the sediment surface the O_2 concentration was about 300 µM in all profiles, corresponding with O_2 saturation. The oxygen concentration appeared to decrease non-linearly with depth. In the upper 0.2 mm the O_2 concentration declined very quickly in all profiles, followed by a gradually lesser steep decline. From day 10 onwards no major changes of micro-profiles were observed, indicating the establishment of steady state. The oxygen diffusion and the oxygen consumption apparently were in balance. At comparable depths, the O_2 concentrations were higher in sediments without nematodes than in sediments with nematodes. Oxygen penetration stabilized at lower depths in the absence of nematodes ($P < 0.05$, table 1) and disappeared, on average, below 1.6 mm in sediments with nematodes, and below 2 mm in sediments without nematodes (Figure 4).

Apparent diffusion coefficients

The apparent diffusion coefficients were calculated for the steady state situation only. For each micro-profile, micro-gradients were calculated over 4 depth intervals: 0-0.15 mm, 0-0.4 mm, 0.05-1.0 mm and from 0.05 onwards to the maximum depth of the oxic zone. Table 2 shows the apparent diffusion coefficients calculated for different depth intervals. The apparent diffusion coefficient depended largely on the depth interval from which it was derived. For each depth interval, however, the values of the diffusion coefficient in treatments with nematodes was higher than in treatments without nematodes. The apparent diffusion coefficients in the control treatments calculated from the depth intervals from 0.05 mm to 1.0 mm and from 0.05 mm to maximal depth were close to the bulk sediment diffusion coefficient, which was obtained by multiplying the porosity ($\phi \approx 0.5$) by the free solution diffusion coefficient ($D_0 = 7.4 * 10^{-6} m^2 h^{-1}$ at 20°C, Broecker and Peng, 1974) and are therefore considered to be the best estimates. Analysis of variance

was carried out on the apparent diffusion coefficients calculated for the 0.05 to 1.0 mm depth interval. The diffusion coefficients in treatments with nematodes were significantly higher than in control treatments ($P < 0.05$, table 1).

Nematodes

The number of nematodes increased from between 50 and 100 individuals per dish at the beginning of the experiment to a mean number of 1010 (s.d. = 196) at the end of the experiment. These 1010 specimens of *D. dievengatensis* represent an estimated biomass of 0.16 mg wet weight.

DISCUSSION

During the transient state the CO_2 production and O_2 consumption rapidly increased and, after a few days, slowly decreased. This pattern was observed by several authors after incubating micro-organisms in a microcosm (Findlay & Tenore, 1982; Abram & Mitchell, 1980; Anderson et al., 1981). In the beginning of the experiment, the high bacterial activity is probably a result of the large quantity of dissolved organic matter (DOM) released from the detritus after wetting the sediment. The DOM forms an immediately available, rich food source for the bacteria and consequently bacterial activity rapidly increases, resulting in high CO_2-production and O_2-consumption. During the transient state the oxygen micro-gradients were very steep and the oxygen was depleted within a few tenths of a millimeter from the sediment surface.

The initial quantity of easy assimilable substrate presumably was exhausted rapidly and, as a result, bacterial activity declined and stabilized at a much lower level. After about 10 days a steady state was established. During the steady state pronounced differences between the treatments with nematodes and without nematodes were observed. In the presence of *D. dievengatensis* 74 % more CO_2 was produced per hour and 74 % more O_2 was consumed per hour, resulting in a 26% higher mineralization of organic carbon over the total period of the

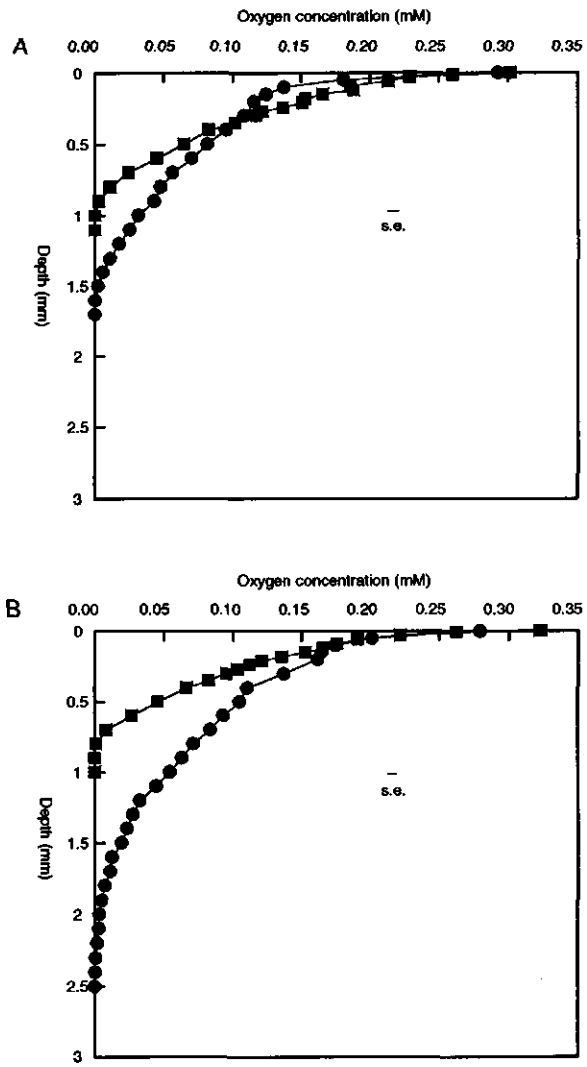

Figure 3: Oxygen microprofiles measured on day 3 (■), in the first period of the experiment and on day 11 (●), in the steady state situation. Fig. 3A in presence of nematodes and fig 3B in absence of nematodes. All points are mean values of 8 measurements. Horizontal bars, denoted by s.e., indicate the mean standard error for all points.

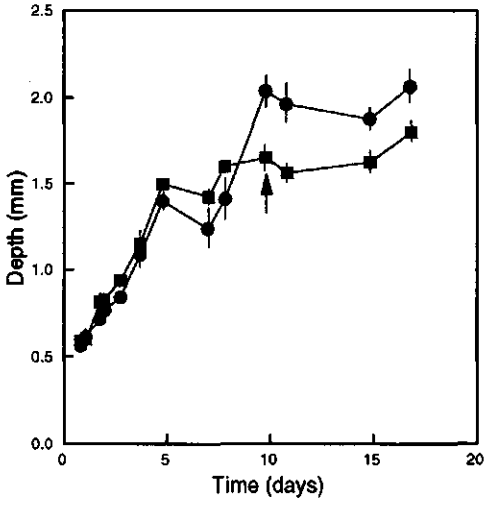

Figure 4: Penetration of oxygen into the sediment during the course of the experiment. (■ sediment with nematodes, ● sediment without nematodes). All points are mean values of 8 measurements. Vertical bars indicate 1 standard error. Arrow head indicates the beginning of the steady state period.

experiment. The diffusion coefficient of oxygen was 40 % - 70 % higher in the presence of nematodes (Table 2), but the maximum penetration depth was lower (Figure 4).

Table 2: Apparent diffusion coefficient ($*10^{-6}$ m^2 h^{-1}) in presence and absence of nematodes calculated for 4 different depth intervals. Figures are mean values over the whole steady state (n=14). (Bulk sediment diffusion coefficient $D_s = 3.6 * 10^{-6}$ m^2 h^{-1})

	without nematodes	with nematodes
Depth interval:		
0.0 to 0.15 mm	0.65 ± 0.19	0.91 ± 0.44
0.0 to 0.40 mm	1.42 ± 0.40	2.13 ± 0.80
0.05 to 1.0 mm	3.17 ± 0.93	5.40 ± 1.60
0.05 to max. depth	4.56 ± 1.65	6.98 ± 1.93

The O_2 consumption and CO_2 production presumably depended almost completely

on the bacterial activity, since the total respiration of the nematodes was only limited. The weight specific respiration of nematodes is 0.029 nmol O_2 h^{-1} µg wet wt^{-1} (Heip et al., 1985). The total wet weight of the nematodes at the end of the experiment was 0.16 mg per dish, on average. So, the estimated respiration of the nematodes in the dishes was 4.6 µmol O_2 $h^{-1}m^{-2}$, which represents only 2% of the total respiration on the last sampling day. The increased O_2 consumption in the presence of nematodes therefore must be the result of the influence of the nematodes on the bacterial population. Effects of nematodes were only detectable during the steady state period. Although the nematode population must have grown during the steady state, they apparently did not affect the steady state as such. Under the conditions of the experiment, the nematodes only changed the level at which the steady state established.

Increased apparent diffusion coefficients are a result of bioturbation (Berner, 1980). The mixing activity of the nematodes thus must have been responsible for the increased diffusion coefficients. To date, only qualitative studies on bioturbation by nematodes have been published (Cullen, 1973; Nehring et al., 1990). Our results provide for the first time quantitative data on bioturbation by nematodes. Apart from mixing of the sediment caused by movements of the nematodes, structures built by nematodes may also enhance diffusion. Some nematode species form a closely spaced network of thread-like intergranular burrows within the surface layer of the sediment (Cullen, 1973). The nematode *Ptycholaimellus sp.* builds membranous tubes from detritus with the aid of mucus (Nehring et al., 1990). Undoubtedly small-scale burrow systems will promote the diffusion of oxygen but the quantitative contribution to oxygen diffusion is unclear. The mechanism by which *D. dievengatensis* increased bioturbation in our study remains to be investigated.

In natural sediments, comparable to the sediment we used, the apparent diffusion coefficients were 1.4 to 18.8 times higher than the bulk sediment diffusion coefficient (Hofman et al., 1991). The lower values were found in sediments with a low density of macrofauna, unknown densities of meiofauna, and low contents of organic matter. We found that in the presence of nematodes alone apparent diffusion coefficients were between 1.4 and 1.7 times higher than in sediments without nematodes, where only molecular diffusion occurred. Nematode densities

were approximately 1000 individuals per 10 cm^2, i.e., similar to densities normally found in marine sediments (Heip et al., 1985). We therefore consider it likely that nematodes will substantially increase bioturbation under field conditions.

In the presence of *D. dievengatensis* higher mineralization of organic carbon was observed. This result is in agreement with the findings of Findlay and Tenore (1982), Rieper-Kirchner (1989) and our earlier findings (Alkemade et al., 1992). The enhanced mineralization in the presence of nematodes may have several possible causes: Grazing of the bacterial population by nematodes may stimulate bacterial activity and therewith the mineralization of detritus (Abram and Mitchell, 1980). A second possiblity is that the production of mucoid substances by nematodes provides a rich additional food source to the bacteria stimulating their activity (Riemann and Schrage, 1978). Finally nematodes may stimulate aerobic bacterial activity by the enhanced diffusion of O_2, either by the movement of pore water and particles or by a burrow system built by nematodes (Abram and Mitchell, 1980; Nehring et al., 1990).

Herman and Vranken (1988) calculated that the bacterivorous nematode *Monhystera disjuncta* had grazing rates of only a few percent per day and therefore was not able to remove senescent cells quickly enough to stimulate bacterial growth. They hypothesized that bioturbation is more important than grazing in stimulating bacterial activity. We cannot assess the exact importance of grazing in our experimental system nor can we exclude the possibility that the presence of mucus to some extent increases the bacterial activity. However, increased bacterial activity by grazing or by the production of mucus will result in higher O_2 consumption, but not in higher diffusion of O_2. The higher O_2 diffusion resulted from the bioturbation due to the nematodes. The most convincing argument for that the enhanced mineralization of organic carbon in the presence of *D. dievengatensis* probably is primarily the consequence of bioturbation is our finding that the increased diffusion on the one hand and the increased oxygen consumption and mineralization of organic carbon on the other hand were of similar magnitude. Grazing and the production of mucus may only have a small additional effect.

CHAPTER SIX

A model for *Spartina anglica* above-ground decomposition and the effects of grazers

R. Alkemade, P.M.J. Herman

ABSTRACT

A model is proposed for evaluating the effects of microbial grazers on *S. anglica*-litter decomposition. The heterogeneity of decomposing litter was described by a number of successive quality classes. Decomposition was considered to be primarily a microbial process. The microbial population was assumed to consist of a number of successional "species" each possessing a preference for a specific quality class. Grazers were included as one population grazing upon the microbial species. Mechanisms by which grazers may stimulate decomposition were evaluated using data from laboratory studies. Removing microbial biomass by grazers had a slight stimulatory effect on the decomposition rate of detritus, but not enough to account for the total effect. Recycling of organic matter by excretion of mucus seemed to have no effect at all. Bioturbation or reworking activity appeared to make the largest contribution to increased decomposition rates.
The model was validated by use of field data. It was shown that the model could describe the loss of carbon from decomposing *Spartina* litter in a large range of field situations. The biomass of bacteria and grazers estimated by the model were much higher than was found in the field. This discrepancy was explained by inaccurate conversion factors and the presence of other decomposers and grazers, not accounted for in the model. This model is potentially useful to evaluate decomposition data from different studies and to calculate an approximate amount of microbes and primary grazers available for higher trophic levels.

INTRODUCTION

Species of the halophyte genus *Spartina* commonly occur in temperate salt marshes over the world. The decomposition of *Spartina* litter has been studied extensively during the past decade. Field studies were carried out to estimate the decomposition rates under varying circumstances (Buth & de Wolf, 1985; Montagna & Ruber, 1982; Valiela et al., 1985 Marinucci et al., 1983; Hemminga & Buth, 1991; Hemminga et al., 1988; Kemp et al., 1990). The effect of microbial grazing on decomposition of *Spartina* was studied in laboratory experiments (Findlay & Tenore, 1982; Alkemade et al., 1992a, 1992b). In these studies it was shown that

decomposition of *Spartina* is primarily a microbial process but can be influenced and stimulated by other organisms (Kemp et al., 1990; Findlay & Tenore, 1982; Abrams & Mitchell, 1980; Alkemade et al., 1992a, 1992b). Several studies indicate that the role of grazers in decomposition processes depends on the resource quality of the substrate. Findlay and Tenore (1982) found a much higher effect of nematodes on the decomposition of *Gracilaria* than on the decomposition of *Spartina*. Rieper-Kirchner (1989) found a stimulating effect of nematodes and of ciliates on the decomposition of seaweed but Alongi and Tietjen (1990) did not find any effect of nematodes on the decomposition of mangrove litter. In an earlier study we found that the nematode *Diplolaimelloides bruciei* did have a stimulating effect on CO_2 release from decomposing fresh leaves, but no effect was found on decomposing older leaves (Alkemade et al. 1992a). The aim of our study was to formulate a model which describes the effect of grazers on decomposing material of different resource quality.

Decomposition of organic matter can be modelled in various ways. Fitting simple regression models to decomposition data is commonly used to describe decomposition (e.g. Buth & de Wolf, 1985; Swift et al, 1979). A number of possible models have been proposed for this purpose but the single exponential decay function is by far the most simple and most frequently used (Kelman Wieder & Lang, 1982). These models are convenient for the description of global decomposition rates but disregard the dynamics of the organisms involved and the continuous change of the composition of the organic matter itself. The single parameter in a negative exponential decay model is a composite result of a multitude of processes and it is impossible to model the influence of e.g. nematode grazing on this parameter.

The dynamics of the decomposition process of *S. anglica* litter can be described more appropriately by using models which include the heterogeneity of the substrate and the dynamics of the microbial populations as well as the dynamics of the grazers. During decomposition of *Spartina* derived detritus the chemical composition changes continuously (e.g. Hemminga et al., 1988; Marinucci et al., 1985). The microbial community present on the detritus may change simultaneously. Newell et al. (1989) found that at the beginning of the decomposition of *S. alterniflora* fungi prevailed, whereas in later phases the bacteria were predominant. Most probably there is a succession of different bacterial species during

decomposition. This assumption was used in our model. All different species may have a preference for a certain class of detritus and all have different growth parameters. The various classes of detritus were described as quality classes sensu Bosatta and Ågren (1991). They defined quality as the substrate accessibility expressed through the growth performance of the microbial community.

Grazers, feeding on the microbial community were included in the model. Meiofaunal grazers may influence the microbial community in various ways. Removing a substantial amount of cells may have a stimulating effect on the microbial population, if the microbial growth is density-dependent and quick enough to replace the removed microbes. If the microbial population does not grow quickly enough the population will be overgrazed leading to decreased decomposition rates (Coleman et al., 1990). Meiofauna may also enrich the substrate of the microbial population by producing mucoid substances which stimulate microbial growth (cf. Riemann & Schrage, 1978). Faecal pellets may have a similar effect. Thirdly, the reworking and bioturbating activity of the fauna may activate the microbial population. Bioturbation increases the oxygen availability in the sediment, whereas fauna, by their reworking activity, may loosen the upper layer of the decomposing litter by mechanical force and herewith enlarge the surface of the substrate on which the microbes attack (Abrams & Mitchell, 1980; Alkemade et al. 1992b). The model was used to investigate the consequences of the various possible mechanisms by which grazers influence decomposition and was validated by use of field data.

The microbial utilization of detritus is considered to be the major process of decomposition and forms the basis of the model. Quality is introduced by dividing the *Spartina* litter in discrete quality classes. By taking only a few classes the model can be easily applied to field data, where a few easily distinguishable quality classes are sampled. Increasing the number of quality classes allows analysis of a quasi-continuum of this character. The model is only implemented for the dynamics of carbon.

THE MODEL CONCEPT

Figure 1 shows the conceptual model in a "flow diagram". A number of discrete quality classes can be distinguished. The recently died-off litter enters the model as first quality litter and moves through the classes until only the most refractory substances remain in the lowest quality class. The detritus of quality i is decomposed in principle by all microbial populations j ($1 \leq j \leq n$). Each population j has its own preference $c_{i,j}$ for litter quality i. If i = j the preference is highest. In the model described here all $c_{i,j} = \varnothing$ if $|i-j| > 1$, i.e. a detrital class i is only decomposed by microbial populations j-1, j and j+1, where j=i. The shift of carbon from quality i to quality i+1 is proportional to the utilization of carbon of quality i by the three microbial populations. Furthermore the detritus of quality i receives the dead microbial cells from the microbial population j = i-1. The living microbial biomass is grazed upon by grazers, for example protozoans or nematodes. As grazers are mobile we assume that the grazers feed on all the microbial populations without preference for certain populations.

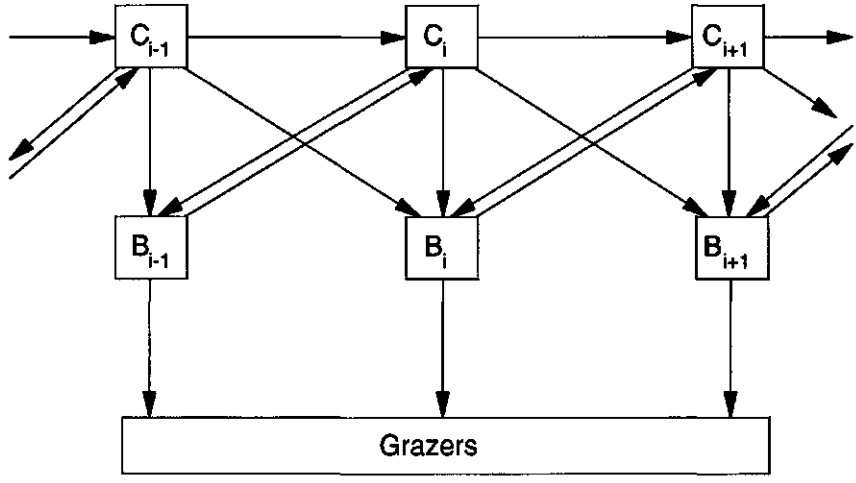

Figure 1: Flow diagram of the model. Symbols are explained in the table 1.

The dynamics of detrital carbon of quality i can be described by the following differential equation:

$$\frac{dC_i}{dt} = -\sum_j U_{i,j} + m_{i-1}B_{i-1} + \alpha_{i-1}\sum_j U_{i-1,j} - \alpha_i \sum_j U_{i,j} \qquad (1)$$

where i denotes the quality classes, i = 1,2,..,n. Quality class 1 is the highest quality detritus and n the lowest. C_i is the amount of carbon of quality class i (g C), $U_{i,j}$ is the utilization rate of carbon of quality i by the microbial 'species' j (g C t^{-1}), m_{i-1} is the mortality rate of the microbial population (t^{-1}), which has a biomass of B_{i-1} (g C), α_i is a dimensionless parameter which describes the shift of carbon from quality i to quality i+1. It is assumed constant for all quality classes, except for the last class where it equals 0. The last class thus is an absorbing state, where the most refractory fraction is kept, and very slowly degraded. However, by changing $\alpha_n > 0$, disappearance (e.g. burial) of refractory detritus can be modelled. In our model α_i (i<n) is chosen so that a certain low fraction of the detritus entering the model remains undecomposed after a certain period.

The change of the biomass of microbial 'species' j is given by the differential equation:

$$\frac{dB_j}{dt} = -m_j B_j - G_j + B_j r_j \left[\frac{K_j - B_j}{K_j}\right] \qquad (2)$$

where B_j is the biomass of the microbial population j expressed as g C, m_j is the mortality rate of B_j (t^{-1}), G_j is the rate of removal of living biomass of population j by the grazers (g C t^{-1}), r_j is the "birth rate" of population j (t^{-1})and K_j is the carrying capacity for the microbial population B_j (g C). A density dependent growth is assumed, which is described by the logistic growth model.

Population j has a choice of three different qualities of substrates: j-1, j and j+1. The availability of carbon for population j depends, therefore, also on the amount of biomass of the other populations present on the different quality classes.

The availability of carbon of quality i for microbial population j per unit of microbial biomass is given by:

$$b_{j,i} = \frac{c_{i,j}C_i}{\sum_j c_{i,j}B_j} \qquad (3)$$

where $c_{i,j}$ denotes the preference of 'species' j for substrate quality i, $b_{j,i}$ has the dimension g C/g C. The preference parameter $c_{i,j}$ is given by:

$$\begin{aligned} c_{i,j} &= c_0, && i=j \\ c_{i,j} &= \frac{(1-c_0)}{2}, && i=j\pm1 \\ c_{i,j} &= 0, && otherwise \end{aligned} \tag{4}$$

So that

$$\sum_i c_{i,j}=1 \tag{5}$$

The total amount of carbon available for 'species' j is given by

$$B_j\sum_i b_{j,i} \tag{6}$$

So K_j is given by:

$$K_j=d_jB_j\sum_i b_{j,i} \tag{7}$$

where d_j denotes the maximal density of 'species' j per unit of substrate available to it.

Considering this, the utilization of substrate of quality i by the microbial 'species' j is given by:

$$U_{i,j}=B_j\frac{r_j}{e_j}\left[\frac{K_j-B_j}{K_j}\right]*\frac{b_{j,i}}{\sum_i b_{j,i}} \tag{8}$$

e_j denotes the trophic efficiency of population j.

The microbial growth and consequently the decomposition is not only dependent on the substrate but also on the grazing activity of micro- and meiofauna present on the *Spartina*-material. G_j in equation 2 denotes the rate of removal of microbial biomass of population j by grazers. The grazers are assumed to be mobile and therefore are able to feed on all microbial 'species' without preference for certain 'species'. The grazers are considered as one population and are present on every

substrate. In chapter 2 an exponential growth equation was derived for nematode populations on two types of decomposing *Spartina* litter. A constant birth rate and a constant rate of disappearance from the litter was sufficient to describe the population growth of the nematodes. The birth rate was found to be equal on both yellow and brown leaves, but the rate of disappearance was higher on brown leaves. We, therefore, assume that the rate of disappearance increases linearly with quality class i. Furthermore it was assumed that the nematode population is limited by the amount of available food and not by the density of the grazers themselves. This type of limitation is described by Michaelis-Menten kinetics. The change of grazers biomass can be described by:

$$\frac{dN}{dt} = -(f*i)*N + Nr_g \frac{\frac{\sum B_j}{N}}{K_g + \frac{\sum B_j}{N}} \qquad (9)$$

N is the amount of grazers present on the substrate, expressed as g C. The parameter f is related to the escape of the animals from the substrate due to flushing, i denotes the mean quality of substrate present, r_g is the birth rate of the grazers, K_g is the microbial biomass per unit of grazer biomass at which the actual birth rate is half the potential birth rate.

G_j, the rate of removal of microbes by the grazers, is then given by

$$G_j = N\frac{r_g}{e_g} * \frac{\frac{\sum B_j}{N}}{K_g + \frac{\sum B_j}{N}} * \frac{B_j}{\sum B_j} \qquad (10)$$

where e_g is the trophic efficiency of the grazers.

In the model all quality classes decompose at different rates. To simulate the different decomposition rates the microbial species need to have different growth parameters, so that the decomposition rate of detritus of quality class 1 is highest and of quality class n is lowest. Obviously the microbial growth rate r_j decreases with increasing quality class i, but also the microbial mortality, m_j, the trophic efficiency, e_j, and the maximal density, d_j, may differ among the different microbial species. The shape of the relations between these parameters and j was assumed

negatively exponential. This implies that the differences between, for example, species 1 and 2 is much greater than the difference between species 9 and 10.
The microbial growth rate r_j of the different species is given by:

$$r_j = r_0 e^{-r_1 * j} \quad (11)$$

where r_0 is the maximal growth rate for the microbial population.
Moorhead & Reynolds (1991) stated that a large proportion of the microbial mortality is growth dependent. We assumed that the microbial mortality m_j depended only on the growth rate of the microbial population. We assumed that when growth rate decreases the mortality rate decreases even more according to equation 12.

$$m_j = (m_0 - m_1 * j) * r_j \quad (12)$$

The trophic efficiency is given by:

$$e_j = e_0 * e^{-e_1 * j} \quad (13)$$

and the maximal density of 'species' j on the available substrate is:

$$d_j = d_0 * e^{-d_1 * j} \quad (14)$$

In table 1 all symbols for the variables used in the equations are summarized, symbols used for the parameters are summarized in table 2.

Table 1: List of variables

C_i	Amount of detritus of quality i (g C)
B_j	Biomass of microbial species j (g C)
N	Biomass of grazers (g C)
$U_{i,j}$	Utilization rate of detrital carbon i by microbes of species j.
G_j	Rate of removal of microbial biomass of population j by grazers
$b_{i,j}$	The amount of detrital carbon of quality i available for a unit of biomass of microbial species j (g C / g C)
r_j	Growth rate of microbial species j
m_j	Death rate of microbial species j
K_j	Carrying capacity for microbial species j
d_j	Maximal density of microbial species j
e_j	Trophic efficiency of microbial species j

Estimation of the model parameters

To evaluate the behaviour of the model in particular situations the number of quality classes and the time unit have to be specified. The number of quality classes may vary from only a few to a large number. We chose 20 classes, so the model describes a quasi continuum. The time unit is 1 hour, since microbial growth may change very quickly. The general model described above was used to evaluate *Spartina* decomposition in both laboratory and field situations. The parameter values used in the model were allowed to vary between ranges which represent realistic values. Table 2 summarizes the parameter ranges set to each parameter.

Table 2: List of parameters and the initial range set for calibration

		Initial range	
		Minimum	Maximum
α_i	the shift of carbon	1.0	3.0
c_0	the preference	0.8	0.8
r_0	maximal growth rate	4.0	6.0
r_i	decrease of growth rate	0.2	0.3
m_0	maximal death rate	0.4	0.5
m_i	decrease of death rate	0.01	0.02
e_0	maximal trophic efficiency	0.2	0.4
e_1	decrease of trophic efficiency	0.1	0.2
d_0	maximal density	0.0005	0.0015
d_1	decrease of maximal density	0.3	0.5
f	flushing rate	0.0004	0.0004
r_g	growth rate of grazers	0.008	0.013
K_g	carrying capacity of grazers	0.5	1.0
e_g	trophic efficiency of grazers	0.15	0.3
N_*	bioturbation parameter	0.005	0.015
Q_{10}	temperature parameter	2.0	2.0

The parameter settings imply that the microbial populations are allowed to grow with growth rate (r_j) ranging from a maximum of 4.9 h^{-1} on the *Spartina* material of the highest quality to a minimum of 0.01 h^{-1} on the lowest quality; the trophic efficiency ranges from 0.59 on the highest quality to 0.054 on the lowest; the

growth dependent mortality rate ranges from 2.43 h^{-1} to 0.0002 h^{-1}; and the maximal microbial densities ranged from 0.0041 g bact. C / g Subs. C to 0.67 * 10^{-6} g bact C / g subs. C on highest quality material and lowest quality material respectively. The preference parameter $c_{i,j}$ describes the ecological amplitude of the microbial species and its value is arbitrarily set to 0.8. The wide ranges of the parameter settings allow a search for the best parameter set fitting to available data. The data from our laboratory experiments on decomposition of *Spartina anglica* were used for the parameter estimations (Alkemade et al., 1992a). CO_2 production of green decaying leaves was measured in presence and absence of *Diplolaimelloides bruciei*, a bacterivorous nematode dominant on decomposing *Spartina* litter. The growth rate of *D. bruciei* is found to be approximately 0.2 d^{-1} at 20 °C or 0.008 h^{-1} (Warwick, 1981; Alkemade et al., 1992a). The range for the growth rate was set from 0.007 - 0.013.The trophic efficiency ranges between 0.15 and 0.3. The flushing rate of the nematodes was estimated in an earlier study and ranged from 0.0002 - 0.0004 (Alkemade et al. in prep.).

Parameter estimation was carried out by calibrating the model on the data, using the computer program package SENECA 1.5 (de Hoop et al., 1992). The calibration procedure in this program is based on simulated annealing, and is adapted to the simultaneous calibration of many parameters in nonlinear coupled differential equation models. For each parameter an initial range is defined on theoretical considerations, and a probability distribution of the parameter within this range is specified. The model is evaluated with a fixed number (in our case: 50) randomly drawn parameter vectors (each containing one value for each of the m parameters to be calibrated) from the parameter space. Goodness of fit of the model for a particular output variable is evaluated as the sum of the squared deviations between model and observational data, standardized with the standard deviation of the data. Model goodness of fit is evaluated as the average variable goodness of fit for the different output variables for which data are available. In this case CO_2 production with and without nematodes were the two series of observations used.

Subsequently the simulated annealing algorithm optimizes the model goodness of fit by drawing new parameter vectors, replacing the worst fitting vector in the set of 50 by any better-fitting new one. As a consequence, the parameter subspace from which the well-fitting vectors are drawn is reduced. For more details we refer to de Hoop et al. (1992).

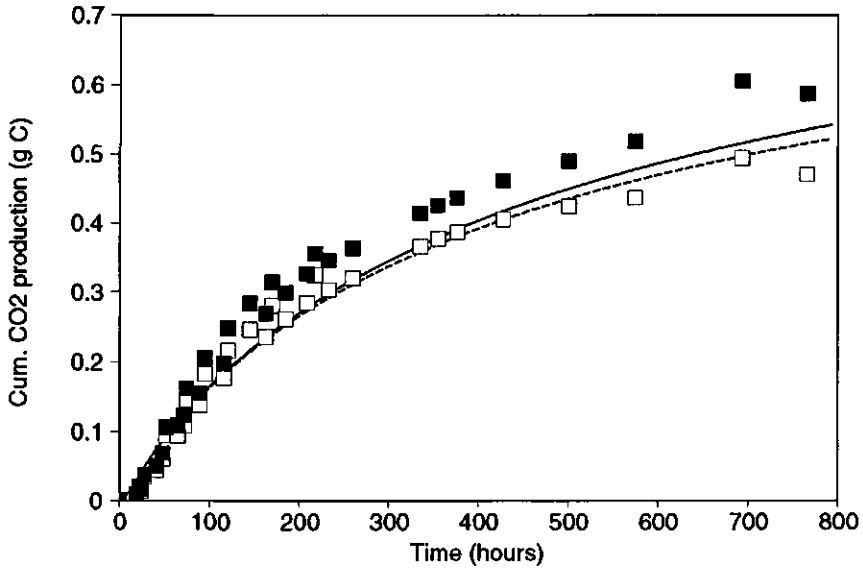

Figure 2: Cumulative CO_2 production of *S. anglica* leaves. Simulated results of the grazing effect. (— simulation in presence of grazers, ■ observations in presence of grazers, --- simulation in absence of grazers, □ observations in absence of grazers)

Table 3: Estimated values of the parameters

α_1	the shift of carbon	2.99
c_0	the preference	0.8
r_0	maximal growth rate	5.79
r_1	decrease of growth rate	0.29
m_0	maximal death rate	0.41
m_1	decrease of death rate	0.016
e_0	maximal trophic efficiency	0.39
e_1	decrease of trophic efficiency	0.12
d_0	maximal density	0.00148
d_1	decrease of maximal density	0.30
f	flushing rate	0.0004
r_g	growth rate of grazers	0.0128
K_g	carrying capacity of grazers	0.50
e_g	trophic efficiency of grazers	0.151
N_*	bioturbation parameter	0.0050
Q_{10}	temperature parameter	2.0

Figure 2 shows the fitted curve for the cumulative CO_2 production as was obtained by calibration. The parameter values are shown in table 3. Grazers only slightly increased the CO_2 production by removing microbial biomass, but not enough to simulate the total effect of the grazers. Figure 3 shows the same model calculated over a longer period of time. In the presence of grazers the cumulative CO_2 production was 5 % higher than in the absence of grazers. The total microbial biomass on the substrate in the presence of grazers is lower than the microbial biomass in the absence of grazers (Fig. 4). The microbial biomass is lower, because the mean quality is lower (thus the maximal microbial density was assumed to be lower) and because the grazers consumed a certain amount.

The total effect of grazers can not be described by grazing alone, apparently other stimulatory factors of grazers are also involved. Two other mechanisms by which nematodes may stimulate decomposition rates are suggested in literature. The excreted mucoid substances and faeces are of high nutritional quality for the bacterial population, and the bacterial growth will be stimulated when these substances are added to the substrate. The second mechanism is bioturbation or reworking the detrital-microbe mixture of grazers. The movements of the animals in the grooves of the leaves may increase the oxygen supply or increase the surface which microbes may attack allowing increased densities of microbial organisms. (Abrams & Mitchell, 1980; Herman & Vranken, 1988). These two mechanisms could easily be implemented in the model by slight modifications.

An expression for the amount of excreted substances is given by subtraction of the amount of microbes removed by the nematodes and the growth of the nematodes. This amount is added to the amount of substrate of the highest quality. In this way the nematode respiration is neglected and the amount added to the substrate is overestimated. To determine the effect of this modification the model was evaluated again and the result is shown in figure 5. The difference between these results and the results obtained by simulating grazing alone is very small, so that the effect of mucus seemed to be very small. The effect of mucus may be limited, since the total amount of mucus produced is very small in comparison with the total amount of substrate.

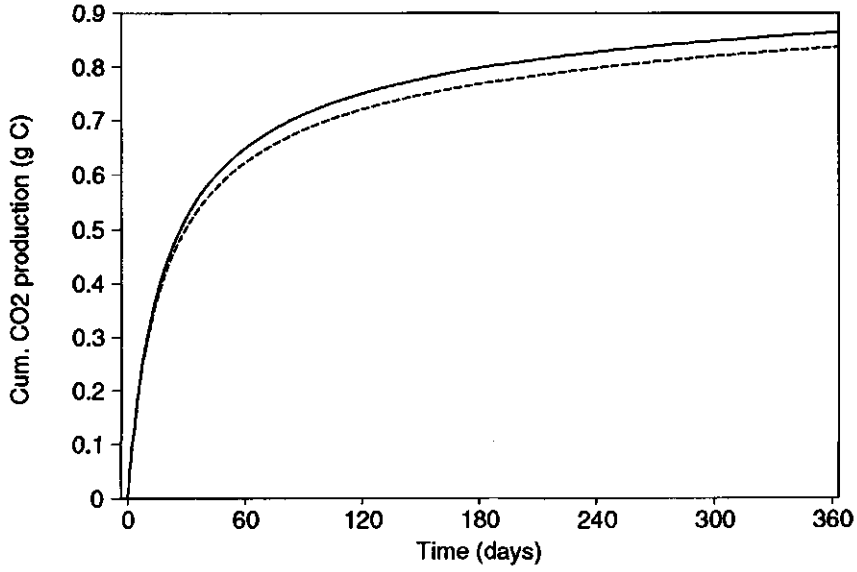

Figure 3: Cumulative CO_2 production of *S. anglica* leaves. Long-term simulated results of the grazing effect. (— simulation in presence of grazers, --- simulation in absence of grazers)

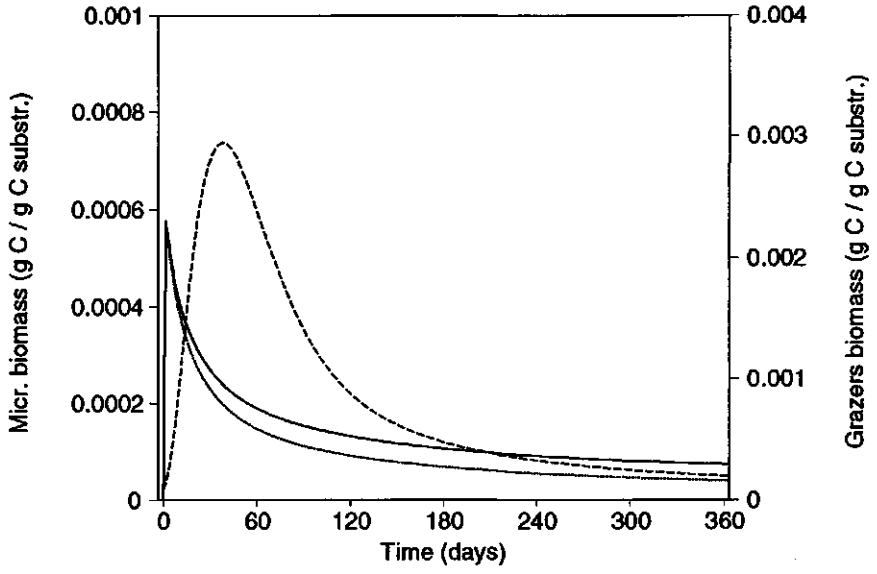

Figure 4: Biomass of the microbial population and the grazers, expressed as g C per g C of substrate in the long-term simulation of the grazing effect. (— simulated biomass of microbes in presence of grazers, ··· simulated biomass in the absence of grazers, --- simulated biomass of grazers)

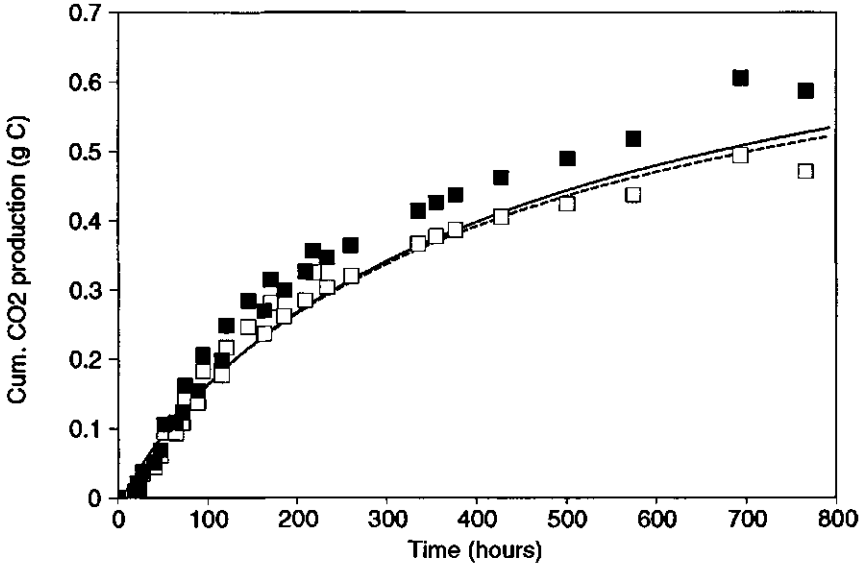

Figure 5: Cumulative CO_2 production of *S. anglica* leaves. Simulated results of the mucus enrichment effect. (— simulation in presence of grazers, ■ observations in presence of grazers, --- simulation in absence of grazers, □ observations in absence of grazers)

The effect of bioturbation or reworking activity is modelled by increasing the maximal density of the microbes on the substrate with a factor proportional to the amount of grazers present. Equation (14) will then become:

$$d_j = (1 + \frac{N}{N_*}) * d_0 * e^{-d_1 * j}$$

where N_* can be interpreted as the nematode density which doubles the maximal microbial density. N_* was estimated in a subsequent calibration run. The bioturbation parameter ranged from 0.005 to 0.015 g C grazers per g C of substrate.

The bioturbation parameter had conspicuous effects on the results. In figure 6 it is shown that bioturbation results in higher CO_2 production in the presence of nematodes. Calculated over a longer period it can be shown that in the first few months a considerable stimulation takes place, but when the decomposition proceeds the effect becomes gradually smaller (figure 7). The microbial biomass relative to the amount of substrate was higher in the presence of nematodes in the

first few months. Later on it was lower than the biomass in the absence of grazers (figure 8). The increase of the microbial biomass may have resulted in an increase of grazers. The increased grazing pressure may consequently decrease the microbial biomass on substrate of lower quality, where the growth of microbes is lower.

Validation of the model.

Three different field data sets were used to validate the model. Firstly the field data summarized by Montagna & Ruber (1980) was used. The weight losses of *S. alterniflora* on three different salt marshes on the east coast of the USA are given in their figure 3. All three experiments started in summer and were carried out during about 1 year. Figure 9 shows the data and the results from our model, with and without grazers. For the simulated results the parameter values, obtained by calibration on laboratory data, were used (table 2). The results indicate that these parameter values fitted quite well. The field data showed a high variation and therefore no conclusive argument could be drawn from the simulated results whether grazers had an effect on decomposition in the field or not.

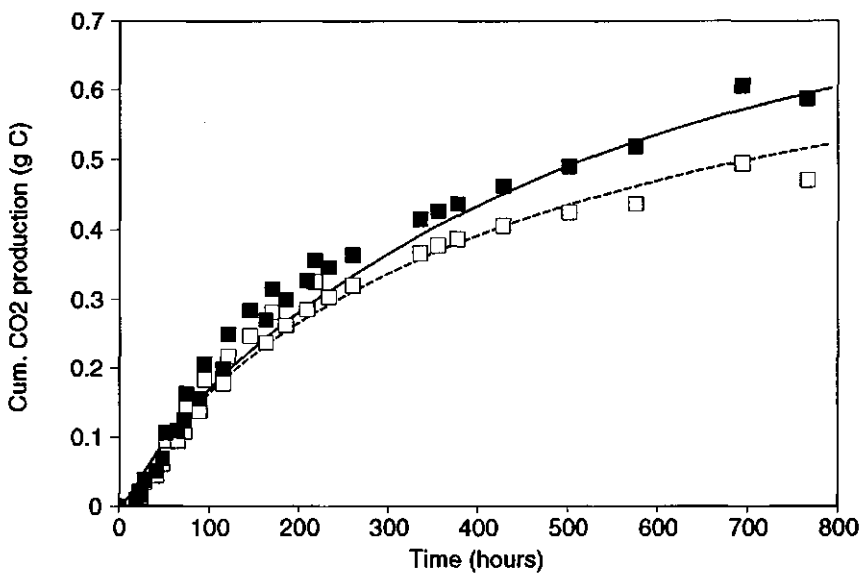

Figure 6: Cumulative CO_2 production of *S. anglica* leaves. Simulated results of the bioturbation effect. (— simulation in presence of grazers, ■ observations in presence of grazers, --- simulation in absence of grazers, □ observations in absence of grazers)

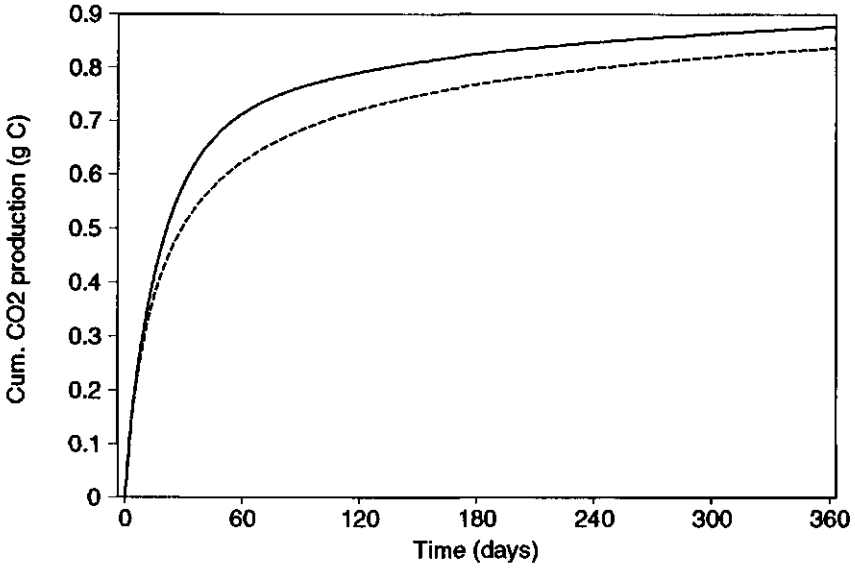

Figure 7: Cumulative CO_2 production of *S. anglica* leaves. Long-term simulated results of the bioturbation effect. (— simulation in presence of grazers, --- simulation in absence of grazers)

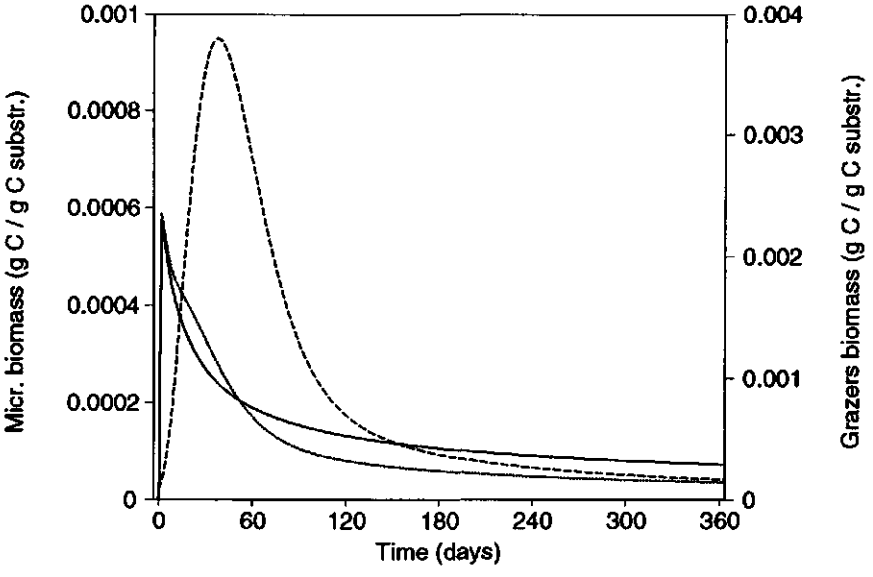

Figure 8: Biomass of the microbial population and the grazers, expressed as g C per g C of substrate in the long-term simulation of the bioturbation effect. (— simulated biomass of microbes in presence of grazers, ··· simulated biomass of microbes in the absence of grazers, --- simulated biomass of grazers)

Montagna and Ruber (1980) also measured decomposition rates of *Spartina alterniflora* in a litterbag study starting in september 1972 in a salt marsh situated in the Parker River Estuary, Massachusetts, USA. In addition they assessed the number of nematodes and bacteria on the decomposing leaves during 1 year. The data set we used was derived from their figures 1 and 2. The conversion from numbers to biomass was done by use of factors obtained from literature. We assumed that 1 bacterial cell had a fresh weight of $5.85 * 10^{-14}$ g (Marinucci et al., 1983), 10 % of the fresh weight was assumed to consist of carbon. The average weight of an individual nematode found in our earlier study, chapter 3, was used to convert the number of nematode into biomass. The carbon content of a nematode was assumed to be 10.6 % of the fresh weight (Heip et al., 1985). As the decomposition in autumn starts much slower than in summer the model needed to be adapted. A temperature effect is needed to describe decomposition in different seasons. A lower temperature induces lower growth rates of microbial organisms and of the grazers. In the model the growth rate of the microbial populations was adapted as follows:

$$r_j = e^{(T-20)*\frac{\ln(Q_{10})}{10}} * (r_0 e^{-r_1 * j})$$

where Q_{10} is the factor by which the growth rate decreases when the temperature decreases with 10 °C, and T is temperature (°C). At 20°C this formulation yields the growth rates calculated from the calibration (table 2). The growth rate of the grazers is adapted similarly:

$$r_g' = e^{(T-20)*\frac{\ln(Q_{10})}{10}} * r_g \qquad (15)$$

A constant temperature of -5°C, during the whole period was assumed and Q_{10} values were 2 for the microbial population and 2 for the grazers. Figure 10 shows the weight losses found by Montagna & Ruber (1980) and the simulated results. In figure 11 the biomass of bacteria and nematodes are shown together with the simulated results. Note that the microbial biomass decreased in the presence of grazers relative to the absence of grazers. During the first few months the model suggested a much higher biomass of gazers and microbes than was found in the field. In later phases of decomposition the simulated results and the biomass found

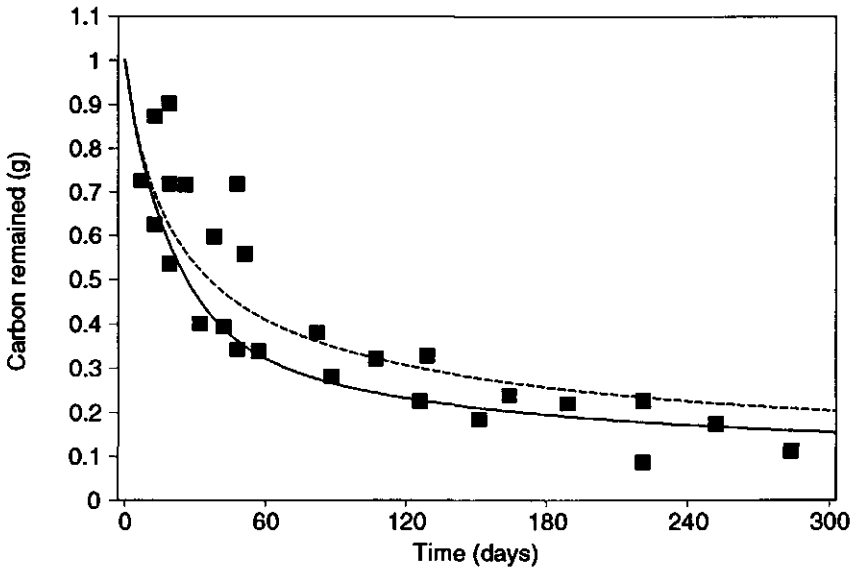

Figure 9: Weight losses of decomposing *S. alterniflora* during summer, expressed as g C. Data derived from Montagna & Ruber (1980). (— simulation in presence of grazers, --- simulation in absence of grazers, ■ = observations)

in the field seemed to be closer to each other, although the field measurements showed a very high variation. This deviation of the model outcome may partly be explained by the inaccurate conversion factors used for calculation of biomass and partly by the presence of other microbial organisms and other grazers on the leaf litter.

The third data set was derived from Buth & Voesenek (1988). They studied the decomposition of leaves and stems of *Spartina anglica* in a Dutch salt marsh. Their study started 29 october 1982 and therefore a temperature effect was also included. Leaves and stems have different quality so the simulation was started with quality class 1 for the leaves and class 3 for stems. In figures 12 and 13 the results for these data are shown, supporting the applicability of the model for a variety of field data.

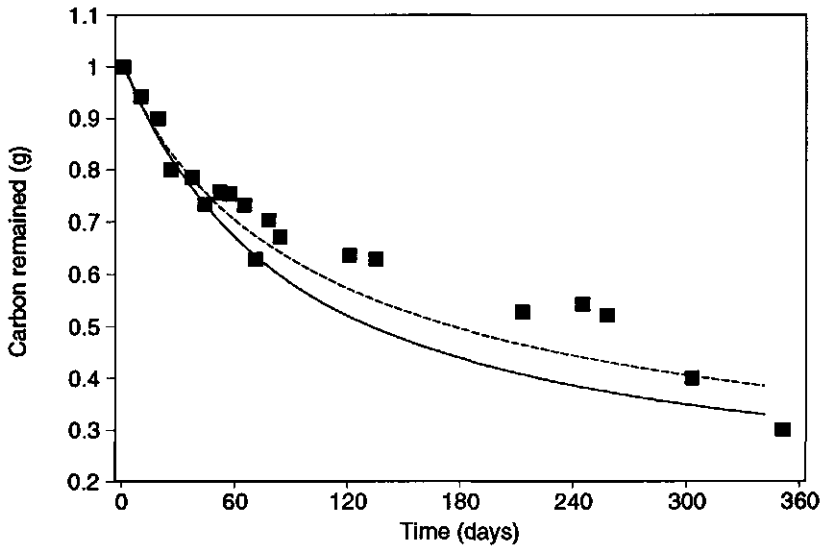

Figure 10: Weight losses of decomposing *S. alterniflora* during winter, expressed as g C. Data derived from Montagna & Ruber (1980). (— simulation in presence of grazers, --- simulation in absence of grazers, ■ = observations)

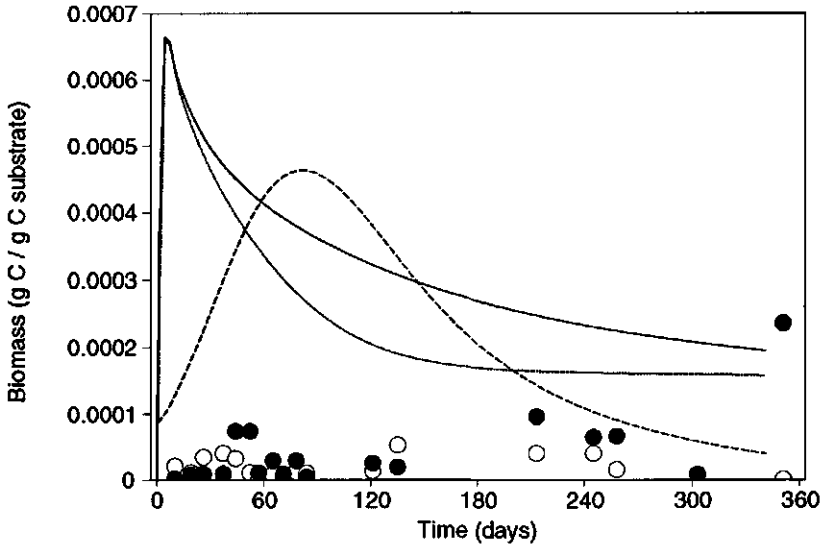

Figure 11: Biomass of the microbial population and the grazers, expressed as g C per g C of substrate of *S. alterniflora*. Data derived from Montagna & Ruber (1980). (— simulated biomass of microbes in presence of grazers, ··· simulated biomass of microbes in the absence of grazers, --- simulated biomass of grazers, ○ = observations of bacteria, ● = observations on nematodes)

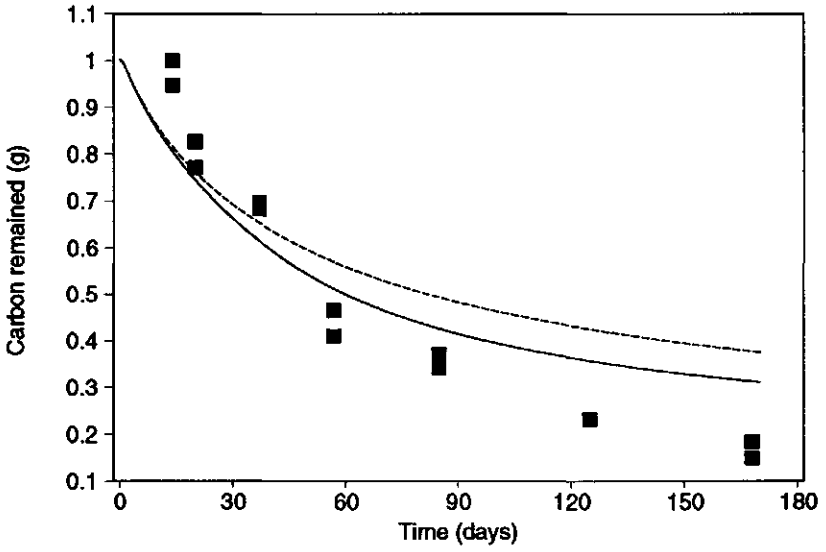

Figure 12: Weight losses of decomposing *S. anglica* leaves, expressed as g C. Data derived from Buth & Voesenek (1988). (— simulation in presence of grazers, --- simulation in absence of grazers, ■ = observations)

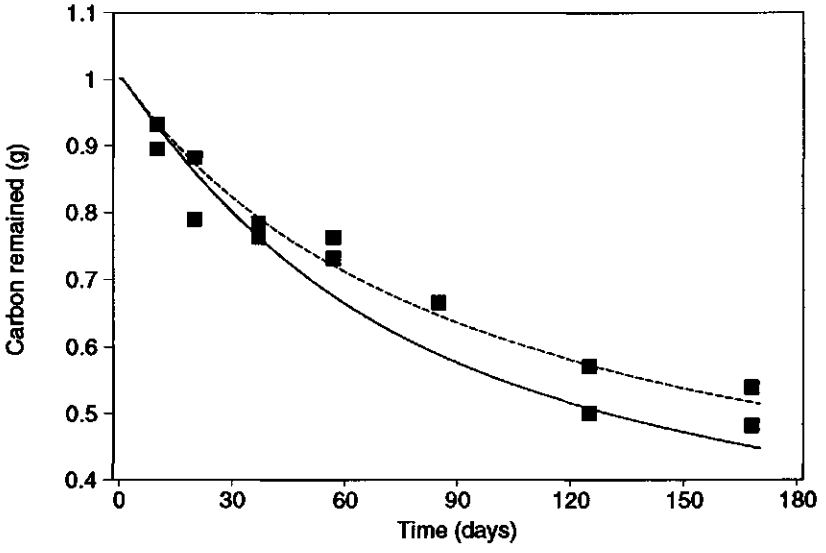

Figure 13: Weight losses of decomposing *S. anglica* stems, expressed as g C. Data derived from Buth & Voesenek (1988). (— simulation in presence of grazers, --- simulation in absence of grazers, ■ = observations)

DISCUSSION

Existing models of the decomposition of organic matter include the dynamics of the microbial population, but higher trophic levels generally are not regarded (Bosatta & Ågren, 1991; Moorhead & Reynolds, 1991). In contrast, food-web models include different trophic levels of the decomposer community but the heterogeneity of the substrate is not accounted for (Hunt et al., 1987; Moore and de Ruiter, 1991). Our model can be considered as a combination of a model describing the decomposition of organic matter and a food-web model with two trophic levels. The combination of both types of models was used since the main goal of our model was to verify the effect of grazers on microbial decomposition of organic matter. The concept of dividing the decomposing litter into quality classes was necessary since different effects were found on substrate of different resource qualities (Findlay & Tenore, 1982; Alkemade et al., 1992a). The quality concept proposed by Bosatta and Ågren (1991) could be used for simulating this quality aspect. Since the effect of grazers on decomposition is a result of the influence of grazers on the microbial populations, the biomass of microbes had to be explicitly calculated.

Three different mechanisms by which grazers may influence decomposition were evaluated using a dataset from laboratory experiments (Alkemade et al., 1992a). In these experiments the effect of the nematode *D. bruciei* on the decomposition of fresh *Spartina anglica* was studied. A considerable increase of CO_2 production was measured in these experiments. The estimated parameters resulting from calibration of the model on these data did describe these data sufficiently well. The resulting parameter estimates were based on a limited number of observations on one of the variables (total CO_2 production). So similar results could be obtained if other parameter values were used. For instance, a lower growth rate combined with a higher microbial density and higher growth rates combined with lower densities result in equal decomposition rates, whereas the population of grazers differed. However, the data to verify the validity of the model were not sufficient to distinguish both situations.

The three mechanisms by which grazers may accelerate decomposition were: (1) the grazing effect; since a maximal density of microbes on the litter was assumed, the removal of cells by grazers enables the microbial population to replace the

cleaned substrate by newly formed biomass on the expense of the substrate (Abrams & Mitchell, 1980); (2) the mucus excretion; grazers may excrete mucoid substances which is a readily available food source for microbes; and (3) bioturbation or reworking activity; interpreted as the physical impact of grazers on the microbial environment leading to changes in microbial activity (Abrams & Mitchell, 1980). The simulation results showed that the removal of microbial biomass by grazers does stimulate decomposition. The acceleration of the CO_2 production found in the laboratory studies, however, could not be described completely. The production of mucus and the excretion of faeces add a small amount of readily available substrate to the decomposing litter. The amount, however, is very small in comparison to the litter so no effects could be observed by simulation. An additional mechanism must be responsible for describing the total effect of the grazers. Bioturbation or reworking may therefore be the major mechanism by which grazers accelerate the decomposition process. This result supports our finding that bioturbation in sediments increased the bacterial activity and therewith the decomposition (Alkemade et al,, 1992b)

If the model was evaluated over a longer period of time, the bioturbating or reworking effects changed from a stimulatory effect to an inhibiting effect. The large population of grazers may then overgraze the slowly growing microbial populations in later phases of decomposition. The higher the resource quality and the bacterial growth the greater the effect of grazers may be. Microbial populations with high potential growth rate may profit from the increased availability of substrate or oxygen generated by the reworking activity of the grazers. Slow growing microbes may not be able to invade empty substrate quickly enough to compensate for the grazing pressure of the grazers. The finding that on older *Spartina* material no effect of grazers was found supports this hypothesis. It might also be possible that the assumption of bioturbation is no longer valid on substrate of lower quality.

The different results obtained for the effect of grazers may be largely explained by the different effects that grazers can have on microbial population with different growth rates. Since the substrate quality determines the growth rate of the microbes, different effects of grazers can be found on material of different resource quality. Findlay & Tenore (1982) found a much higher effect on *Gracilaria* detritus than on the more recalcitrant *Spartina* litter. On mangrove litter no effects were

found (Tietjen and Alongi, 1990). We found only a clear effect on fresh *S. anglica* litter and not on older material (Alkemade et al., 1992a).

The validity of the model was also tested using a number of different data sets. The model, calibrated on the results of laboratory experiments, could be used to describe a variety of field data. The decomposition rates found in field situation could be described by the model. However, the relatively small effect of grazers could not be found in the field. Most of the data showed a high variability so that the goodness of fit found in the presence of grazers was not higher than in the absence of grazers. To verify the validity of the model accurate and simultaneous measurements of the microbial biomass, grazer biomass and decomposition rates are needed.

SUMMARY

Introduction

Salt marshes in temperate regions are very productive natural vegetations. These vegetations frequently reach an above-ground production of more than 1 kg of dry weight per m^2 per year. Herbivores consume only a small proportion of the annual plant production. Almost the entire amount of above ground plants dies after senescence. A small proportion may be washed away by the tides, but the major part remains at the salt marsh where it decomposes in the canopy or at the sediment surface.
Dead plant material is primarily decomposed by micro-organisms, such as fungi and bacteria. The chemical composition of the detritus to a large extent determines the rate of decomposition. A number of abiotic factors, such as temperature and humidity, also influence the decomposition process. In addition the process may be affected by fauna, present on the decomposing plant material.

In this thesis the role of nematodes in decomposition of *Spartina anglica* was studied. This plant species commonly occurs in salt marshes of Western Europe. In addition, one chapter is dedicated to the association between nematodes and decomposing seaweed in a completely different habitat: an Antarctic beach. In the first part of this thesis the relation between decomposition and naturally occurring nematode populations is studied. This part consists mainly of field studies. Nematodes, which are associated with the decomposition process are identified, and the population dynamics of one of these species is studied in detail. The second part of this thesis is dedicated to laboratory and model studies which were carried out to investigate the effects of nematodes on decomposition of *S. anglica* detritus and the possible mechanism underlying these effects.

Nematode populations on decomposing plant material

Nematodes are abundant on both *S. anglica* litter and on stranded Antarctic seaweed. We found that on standing dead *Spartina anglica* plant parts the nematode population frequently reached densities of 3000 individuals per g DW. When leaf material on the sediment surface was investigated even much higher

nematode densities were found, up to 47,000 individuals per g DW. At Antarctic beaches nematode densities up to 26,000 individuals per g DW were found on seaweed wrack.

Although numerous, not all of these nematodes present on plant detritus are expected to influence the decomposition process. In chapter I an attempt was made to distinguish the nematode species which play a role in decomposition of *S. anglica* detritus from nematodes which do not have such a role. As decomposition is largely a microbial process, higher decomposition rates presumably coincide with a higher microbial production and, consequently, a higher availability of food for microbivorous nematodes. Amongst the microbivorous nematodes, those species were considered of possible importance to the decomposition process when their numbers increased with increasing decomposition rate. In the experiments, mesh containers, filled with *Spartina anglica* leaves, were placed on the sediment surface. Different decomposition rates were induced by using decaying leaf material of different ages and by repeating the experiments during four subsequent seasons. Mesh containers with inert material (plastic drinking straws) served as controls. Sixty nematode species were found in the mesh containers. Using a multivariate analysis (redundancy analysis) different nematode communities were found on plant material with different decomposition rates. These differences were caused by the changing abundance of only a few species. The majority of the species were found in equal numbers in treatments with decomposing *Spartina* leaves and in the control treatment. The numbers of individuals of those species which appeared closely correlated with the decomposition rate of *Spartina anglica* leaf-detritus were all bacterivorous nematodes. Numerically the most dominant were species of the family Monhysteridae (*Diplolaimelloides bruciei, Diplolaimella dievengatensis, Monhystera parva*). The highest numbers of these nematodes were found in treatments with the highest decomposition rates i.e. on decaying fresh leaves, during the warmer seasons. In the winter, when decomposition is slower, their numbers were lower.

The species diversity on standing dead plant parts of *Spartina anglica* is much lower than the species diversity on the sediment surface in mesh containers filled with *S. anglica* leaves. The dominant species on standing dead plants are the bacterivorous nematodes *Diplolaimelloides bruciei, Monhystera disjuncta*

and *Pellioditis marina*. In chapter II a study is presented on the population dynamics of *D. bruciei*. This species was commonly found on above ground plant parts of *Spartina*. In a field study, population densities of this species were estimated on four classes of *S. anglica* plant material, representing the whole range of decomposition stages found in the canopy. *D. bruciei* was found throughout the year on all types of plant material, including living green plant parts. The population densities were highest on the older plant material, where densities of 1000-2000 individuals per g DW were reached. The highest densities were recorded in late summer and autumn.

S. anglica vegetations are regularly flooded at high tide, which potentially reduces the nematode population density on the plant material, as nematodes may be flushed from the plants. Since in situ measurements of the flooding effect are not possible, the population dynamics of *D. bruciei* was studied in the laboratory under a controlled flooding regime. The population densities of *D. bruciei* indeed seemed to be highly influenced by flooding. A considerable part of the population disappeared during flooding, but on younger, yellow, decomposing leaves the rate of removal by flushing was much lower than on older, brown, leaves. This is probably caused by the change of the leaf structure during decomposition. Nematodes may become less well attached to the leaf surface when the groove structure of the leaves disappears with progressive decay; consequently, a higher proportion is flushed away. The growth rate of the population, however, was equal on both leaf types. The growth rate of the nematode population, as estimated in the laboratory, was used to calculate the total production of nematodes in the field. It was shown that the total biomass production of *D. bruciei* equalled 114 mg C per m^2 per year. If 30% of the detritus was decomposed by bacteria and a trophic efficiency of 10 % is assumed, the total amount of bacterial carbon ingested by *D. bruciei* accounted for 7.5 % of the total bacterial biomass produced. It was estimated that the dominant bacterivorous nematodes together may consume over 20% of the total bacterial biomass production.

In chapter III a study of nematodes found in stranded seaweed at an Antarctic beach is presented. Large amounts of seaweed are deposited along the coast of Admiralty Bay, King George Island, Antarctica. The stranded seaweed partly decomposes on the beach and supports populations of various meiofauna

species, mostly nematodes. The factors determining the number of nematodes found in the seaweed packages were studied. The densities of nematodes appeared to be correlated primarily with salinity, height and C:N ratio of the detritus. Salinity and height were most likely related to the flooding regime in conjunction with the off-stream of melt water. Decomposition rate appeared mainly determined by the water content and the sediment composition. Melt water run-off or the impact of the surf probably increased seaweed weight losses in these situations.

The effect of nematodes on decomposition of *S. anglica*

Experiments with *D. bruciei*, a species numerously present on standing dead *S. anglica* plants (see chapter II), were set up to study the effect of this nematode on decomposition (chapter IV). Green and yellow leaves were placed on agar in petri dishes and inoculated with *D. bruciei*. CO_2 production was determined regularly after inoculation. Weight, carbon and nitrogen losses were determined at the end of the experiment, 30 days after inoculation. In the presence of nematodes, CO_2-production on green, decaying leaves increased by 20 - 25 %. Losses of dry weight, carbon and nitrogen during decomposition increased with at least 30 %. On yellow, more senescent leaves no effect on CO_2-production was found, but losses of dry weight, carbon and nitrogen tended to be higher in the presence of nematodes. The results of this study show that *D. bruciei* may enhance the decomposition rate of *S. anglica*-leaves; the extent of the stimulatory effect, however, depends on leaf condition and the population density of the nematode. The minimal nematode population density for a measurable stimulatory effect was estimated to be 4000 individuals per g DW of *S. anglica* leaves. As described in chapter II, field population densities are often of the same order of magnitude.

A part of the senescent *S. anglica* leaves and stems decompose at the sediment surface, where the material is covered with sediment. In chapter I a clear correlation was found between the number of the bacterivorous nematode *Diplolaimella dievengatensis* and the decomposition rate of *S. anglica* detritus present on the sediment surface. The effect of the *D. dievengatensis* on the carbon mineralization of *S. anglica* detritus was examined in a laboratory

experiment (chapter V). Detritus mixed with sediment appeared to decompose at higher rates in the presence of the nematodes. CO_2 production per hour was 74 % higher in the presence of the nematode than in its absence; O_2 consumption per hour increased to a similar extent. Diffusion coefficients were calculated from measurements of both O_2 consumption, using gas chromatography, and O_2 micro-gradients, using micro-electrodes. The apparent diffusion coefficient of O_2 in the sediment in the presence of nematodes was 40% to 70 % higher than the bulk sediment diffusion coefficient. Since the increase of the CO_2 production and of the diffusion of oxygen in the presence of nematodes was of the same magnitude, we concluded that the enhanced turnover time of *Spartina* detritus presumably was largely caused by the bioturbation activity of the nematodes.

A simulation model was constructed to quantify the relations between decomposing *S. anglica* detritus, bacteria and their grazers (chapter VI). The model takes the various stages of above ground litter decomposition into account. The heterogeneity of the decomposing litter was described by a number of successive quality classes. Decomposition was considered to be primarily a microbial process. The microbial population was assumed to consist of a number of successional species each possessing a unique preference for the different quality classes. Grazers were all considered as a single species grazing upon all microbial species. Three mechanisms by which grazers may stimulate decomposition were evaluated using the data from the laboratory study presented in chapter IV. In the first place: if the microbial population grows to a certain maximal density than removing microbial biomass by grazers may stimulate decomposition since space is created for growth of new microbes at the expense of organic substrate. In the second place: the excretion of highly nutritive mucus by grazers may stimulate bacterial growth. In the third place: reworking of the sediment-detritus-microbial mixture in the grooves of the leaves (see also chapter II), or in the upper layer of the sediment may increase the oxygen availability and may, by mechanical force, enlarge the surface of the substrate on which the microbes attack. The model calculations suggested that removing of microbial biomass by grazers has some stimulatory effect on the decomposition rate of detritus, but not enough to account for the total effect. Recycling of organic matter by excretion of mucus seemed to have no effect at all.

According to the model, bioturbation or reworking contributed most to the stimulation of the decomposition rate.

The model was validated with field data. The model could describe field data obtained from a variety of locations. The biomass of bacteria and grazers estimated by the model were in the same order of magnitude as those found in the field. The model is useful to evaluate decomposition data from different studies and calculate an approximate amount of microbes and primary grazers available for higher trophic levels.

When the model calculations were performed over a period of about a year the stimulating effect of grazers gradually seemed to vanish. This is in agreement with the experiments described in chapter IV, which show that the effect of nematodes on decomposing yellow leaves were less pronounced than on green leaves. Thus, any stimulatory effect of nematodes on decomposition of *Spartina anglica* in the salt marsh may be restricted to the first stages of the decomposition process.

SAMENVATTING

Inleiding

In gematigde gebieden behoren schorren en kwelders tot de meest produktieve natuurlijke vegetaties. De primaire produktie van deze vegetaties bedraagt vaak meer dan 1 kg droge stof per m^2 per jaar. In deze vegetaties komt begrazing door herbivoren nauwelijks voor. Vrijwel alle bovengrondse plantedelen sterven daarom af na de veroudering. Een klein deel van dit afgestorven plantemateriaal kan worden meegenomen door getijde stromingen, maar het overgrote deel blijft op het schor aanwezig en wordt ter plaatse afgebroken.
Het achtergebleven plantemateriaal wordt afgebroken door schimmels en bacteriën. Er zijn verschillende factoren die de snelheid van het afbraakproces kunnen beïnvloeden. Over het algemeen bepaalt de chemische samenstelling van het plantemateriaal voor een groot deel de afbraaksnelheid, maar ook de temperatuur en de vochtigheid zijn belangrijke factoren. De op het plantemateriaal aanwezige fauna kan de afbraaksnelheid eveneens beïnvloeden.

In dit proefschrift is een studie gemaakt naar de rol die nematoden kunnen spelen in het afbraak proces van *Spartina anglica*, een grasachtige plant die veel voorkomt op de schorren van West Europa. Daarnaast is één hoofdstuk gewijd aan de nematoden die voorkomen op aangespoeld zeewier in een totaal andere omgeving: een strand op King George Island, Antarctica. In het eerste deel van het proefschrift wordt ingegaan op de samenhang die er bsestaat tussen afbraak van plantemateriaal en nematodenpopulaties. De populatie dynamiek van één van de nematodensoorten, die een duidelijke relatie vertonen met afbraak, *Diplolaimelloides bruciei*, is in meer detail bestudeerd. Dit gedeelte van het proefschrift bestaat voor het grootste deel uit de resultaten van veldonderzoek. Het tweede deel van dit proefschrift is gewijd aan laboratorium- en modelstudies, uitgevoerd om de invloed die nematoden hebben op de afbraak van *S. anglica* te onderzoeken. Ook de mogelijke mechanismen, waarmee nematoden de afbraak kunnen beïnvloeden, zijn daarbij meegenomen.

Nematodenpopulaties op plantemateriaal in afbraak

Op zowel *Spartina anglica* materiaal in afbraak als op aangespoeld Antarctisch

zeewier zijn nematoden talrijk . Op dode plantendelen in het gewas van *S. anglica* bereikt de nematodenpopulatie veelvuldig dichtheden van meer dan 3000 exemplaren per gram drooggewicht. Dichtheden tot 47.000 individuen per gram drooggewicht werden gevonden op bladmateriaal dat op het sediment was neergelegd. Op het zeewier langs een strand in Antarctica komen dichtheden tot 26.000 individuen per gram droog gewicht voor.

Hoewel erg talrijk, wordt niet verwacht dat alle nematodensoorten die gevonden worden op het plantemateriaal invloed hebben op het afbraak proces. In hoofdstuk I wordt getracht om nematodensoorten, die een rol kunnen spelen bij de afbraak van *S. anglica*, te onderscheiden van soorten, die een dergelijke rol niet spelen. Omdat afbraak voornamelijk een microbieel proces is, zullen hogere afbraak snelheden gepaard gaan met hogere microbiële produktie, waardoor meer voedsel beschikbaar komt voor nematoden die zich voeden met microben. Er werd vanuit gegaan dat slechts dié microbivore soorten van belang zijn bij de afbraak,waarvan de aantallen toenamen met toegenomen afbraaksnelheden. Bakjes, aan boven- en onderkant afgesloten met gaas en gevuld met *S. anglica* bladeren, werden uitgelegd op het schorsediment. Verschillende afbraaksnelheden werden verkregen door gebruik te maken van bladeren in verschillende stadia van afbraak. De experimenten werden herhaald gedurende verschillende seizoenen. Bakjes met inert materiaal (plastic rietjes) werden gebruikt als controle behandeling. In de bakjes werden 59 nematodensoorten aangetroffen. Door gebruik te maken van een multivariate analyse methode ("redundancy analysis") konden op bladeren met verschillende afbraaksnelheden nematodengemeenschappen met een verschillende soortensamenstelling worden onderscheiden. De verschillen in soortensamenstelling werden vooral veroorzaakt door veranderingen van dichtheden van een klein aantal soorten. Van de meeste soorten werden ongeveer evenveel exemplaren gevonden in de behandelingen met plantemateriaal als in de controle behandeling. De soorten, waarvan het aantal duidelijk gecorreleerd was met de afbraaksnelheid, waren allen bacterivore soorten. Drie van deze soorten behoren tot de familie van Monhysteridae (*Diplolaimeloides bruciei, Diplolaimella dievengatensis, Monhystera parva*). De hoogste aantallen van deze soorten werden gevonden in de behandeling waarin de hoogste afbraaksnelheden gemeten werden, namelijk de verse bladeren, gedurende de warmere seizoenen. In de winter, wanneer de afbraaksnelheden

laag zijn waren de aantallen van deze soorten ook laag.
De soortsdiversiteit, die werd gevonden op dode plantedelen in het gewas van *S. anglica*, is veel lager dan die in de gazen bakjes op het sediment. De zich met bacteriën voedende soorten *Diplolaimelloides bruciei*, *Pellioditis marina* en *Monhystera disjuncta* zijn de meest dominante soorten die voorkomen op plantedelen in het gewas. In hoofdstuk II wordt ingegaan op de populatie dynamiek van *D. bruciei*. In een veldstudie werd een schatting gemaakt van de populatie dichtheden van deze soort op vier verschillende klassen van bovengronds *S. anglica* plantemateriaal. De vier klassen bestreken alle stadia van het afbraak proces in het gewas. *D. bruciei* werd het hele jaar door gevonden op alle typen bovengronds plantemateriaal, dus zowel op het levende groene blad als op afgestorven stengeldelen. De hoogste dichtheden kwamen voor op het oudere plantemateriaal waar dichtheden werden bereikt van 1000 tot 2000 individuen per gram drooggewicht, althans in de zomer en herfst.
S. anglica vegetaties overstromen regelmatig bij hoog water. Omdat nematoden dan kunnen worden meegenomen door de vloed, heeft overstroming mogelijk een verlaging van de dichtheden tot gevolg. Het effect van overstroming kan onmogelijk in het veld gemeten worden. Daarom is in het laboratorium de populatie dynamiek van *D. bruciei* bestudeerd met een kunstmatig bevloeiingssysteem. Overstroming had een duidelijk effect op de populatiedichtheden van *D. bruciei*. Veel dieren spoelden weg bij het onder water zetten van het bladmateriaal, maar van jongere, gele, bladeren werden er naar verhouding minder dieren weggespoeld dan van oudere, bruine bladeren. Dit komt waarschijnlijk door het verschil in oppervlaktestructuur van deze bladeren. Nematoden kunnen wegkruipen in de groeven van de bladeren, waardoor ze bij hoog water niet worden weggespoeld. Gedurende het afbraakproces vallen bladeren uiteen in loshangende draden, waar nematoden zich niet langer op kunnen vasthouden, met als gevolg dat er meer nematoden worden weggespoeld. De groeisnelheid van de populatie was echter gelijk op beide typen bladmateriaal. Met behulp van de groeisnelheid, bepaald in het laboratorium, werd de totale produktie van de nematodenpopulatie in het veld berekend, welke geschat werd op 114 gram koolstof per m^2 per jaar. Als nu 30 % van het plantemateriaal door bacteriën wordt afgebroken en deze een omzettingsefficiëntie hebben van 10 % dan zal ongeveer 7,5 % van de bacteriële biomassa, die gevormd is op *S. anglica*, door

D. bruciei worden geconsumeerd. Alle bacterieëtende nematoden tezamen zouden dan meer dan 20 % van de geproduceerde bacteriële biomassa kunnen consumeren.

Een studie naar de nematoden die voorkomen op aangespoeld zeewier op een Antarctisch strand wordt gepresenteerd in hoofdstuk III. Grote hoeveelheden zeewier spoelen aan langs de kust van Admiralty Bay, King George Island, Antarctica. Het zeewier wordt gedeeltelijk afgebroken op het strand en herbergt dan verscheidene soorten dieren, vooral nematoden.

De factoren die het aantal nematoden in deze zeewierpakketten bepalen werden bestudeerd. De dichtheden bleken vooral af te hangen van het zoutgehalte van het zeewier, de hoogte waar het zeewier op strand was terecht gekomen en de koolstof-stikstof verhouding van het zeewier. De hoogte en het zoutgehalte zijn gerelateerd aan de mate waarin de vloed een zeewierpakket bereikt en aan de aanwezigheid van smeltwaterstroompjes. De afbraaksnelheid bleek vooral af te hangen van het watergehalte van het zeewier en van de samenstelling van het sediment, waarmee het zeewier vermengt was. Zowel het stromende smeltwater als het zeewater die het zeewier overspoeld hadden mogelijk een verhoogde gewichtsafname van het zeewier tot gevolg.

Het effect van nematoden op de afbraak van *S. anglica*

Om het effect op de afbraak te bestuderen van *D. bruciei*, een soort die veel voorkomt op afgestorven *S. anglica* plantemateriaal werden experimenten opgezet (zie hoofdstuk II). Deze studie wordt weergegeven in hoofdstuk IV. Groen en geel blad werden op petri schaaltjes, gevuld met agar, gelegd en geïnoculeerd met *D. bruciei*. Na deze inoculatie werd regelmatig de CO_2-produktie gemeten. Gewichtsverlies en het verlies van koolstof en stikstof werden bepaald aan het einde van de experimenten. In de aanwezigheid van nematoden was de CO_2-produktie van de aanvankelijk groene, in afbraak zijnde bladeren 20 - 25 % hoger dan in afwezigheid van deze dieren. Het verlies aan drooggewicht, aan koolstof en aan stikstof was minstens 30 % hoger. Op gele, wat meer verouderde bladeren was geen effect van de nematoden op de CO_2-produktie waarneembaar, maar het verlies aan drooggewicht, koolstof en stikstof leek wel hoger te zijn in aanwezigheid van de nematoden. Met deze resultaten

werd aangetoond dat *D. bruciei* in staat is de decompositiesnelheid van *S. anglica* te verhogen. De mate waarin lijkt af te hangen van de eigenschappen van het blad en van de populatiedichtheid van de nematoden. De minimale dichtheid van nematoden voor een meetbaar stimulerend effect werd geschat op 4000 exemplaren per gram drooggewicht. Zoals beschreven in hoofdstuk II komen dichtheden van dezelfde orde van grootte regelmatig voor in het veld.

Een deel van de afgestorven *S. anglica* bladeren en stengels breken af op het schorsediment. Dit materiaal wordt dan gedeeltelijk bedekt met sediment. In hoofdstuk I werd een duidelijke correlatie gevonden tussen het aantal individuen van de bacterivore nematode *Diplolaimella dievengatensis* en de afbraaksnelheid van *S. anglica*. Het effect van deze nematodesoort op de koolstofmineralisatie van *S. anglica* detritus werd onderzocht in een laboratoriumexperiment (hoofdstuk V). Plantemateriaal, gemengd met sediment, bleek een hogere afbraaksnelheid te vertonen in de aanwezigheid van nematoden dan in de afwezigheid van die dieren. De CO_2-produktie per uur bleek in de aanwezigheid van nematoden 74 % hoger te zijn dan hun afwezigheid. Ook de O_2-consumptie werd in gelijke mate verhoogd. Diffusiecoëfficiënten van O_2 werden berekend uit metingen van zowel de O_2-consumptie (met behulp van gaschromatografie) als de O_2-microgradiënten in de toplaag van de bodem (gemeten met behulp van microëlektroden). De schijnbare diffusiecoëfficiënt van O_2 in het sediment in aanwezigheid van nematoden was 40 - 70 % hoger dan de bulk-sediment-diffusiecoëfficiënt. Omdat de toename van de CO_2-produktie en de toename van de diffusie van O_2 in de aanwezigheid van nematoden in de zelfde orde van grootte ligt kon geconcludeerd worden dat de toegenomen omzettingssnelheid van *Spartina* materiaal hoogstwaarschijnlijk voor het grootste gedeelte moet worden toegeschreven aan de bioturbatie van nematoden.

Een simulatiemodel werd opgesteld om de relaties tussen de afbraak van *S. anglica* materiaal, de bacteriën en hun grazers te kwantificeren (hoofdstuk VI). In het model wordt rekening gehouden met de verschillende stadia van bovengrondse afbraak. Deze heterogeniteit van het materiaal werd beschreven door het definiëren van een aantal opeenvolgende kwaliteitsklassen. De microbiële populatie werd gezien als een aantal soorten die elkaar successievelijk opvolgen, elke soort heeft een specifieke preferentie voor één van de kwaliteitsklassen. Grazers werden beschouwd als één enkele populatie die alle microbiële soorten

zonder voorkeur consumeert. Met behulp van het model werden drie mechanismen, waarmee grazers de afbraak kunnen stimuleren, doorgerekend. De eerste betrof het volgende: wanneer de microbiële populatie tot een bepaalde maximale dichtheid kan groeien, dan kan het consumeren van microbiële biomassa een stimulering tot gevolg hebben, omdat er ruimte wordt gecreëerd voor de groei van nieuwe microben ten koste van het organisch substraat. In de tweede plaats kan excretie van gemakkelijk afbreekbare stoffen, zoals mucus, door grazers de bacteriële groei stimuleren. In de derde plaats: het omwerken van het mengsel van sediment, detritus en bacteriën in de groeven van de bladeren (zie ook hoofdstuk II), of in de bovenste laag van het sediment kan de zuurstofbeschikbaarheid verhogen of, door de mechanische werking, het oppervlak van het substraat, waarop bacteriën kunnen leven vergroten. De modelberekeningen wijzen erop dat het wegnemen van microbiële biomassa door grazers wel een stimulerend effect kan hebben, maar dat het niet het totaal gevonden effect kan verklaren. Excretie van mucus lijkt in z'n geheel geen effect te hebben. Het omwerken, of de bioturbatie, lijkt de belangrijkste bijdrage aan de verhoogde afbraaksnelheid te leveren.

Het model werd gevalideerd met veldgegevens. Het model kan decompositiegegevens afkomstig van een groot aantal veldstudies redelijk beschrijven. Modelschattingen van de biomassa van zowel bacteriën als grazers waren in dezelfde orde van grootte als gemeten in het veld. Het model is bruikbaar bij het doorrekenen van gegevens over afbraakprocessen en levert schattingen op voor de microbiële biomassa en die van de grazers.

Wanneer de modelberekeningen worden uitgevoerd over een periode van ongeveer 1 jaar dan verdwijnt geleidelijk het stimulerende effect van de grazers. Dit lijkt in overeenstemming te zijn met de experimenten beschreven in hoofdstuk IV, waarin werd aangetoond dat het effect van nematoden op de afbraak van ouder materiaal minder was dan op groen bladmateriaal. Dus, enig stimulerend effect van nematoden op de afbraak van *S. anglica* op het schor zal beperkt zijn tot de eerste stadia van het afbraakproces.

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