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Raíssa Vieira Corrêa

Resposta dos nematódeos de praias arenosas frente ao impacto das mudanças climáticas: diversidade taxonômica e funcional

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas (Biodiversidade Neotropical) da Universidade Federal do Estado do Rio de Janeiro como requisito para obtenção do grau de Mestre em Ciências Biológicas.

Orientadora: Profª. Drª. Tatiana Fabricio Maria
Coorientador: Prof. Dr. Ricardo Silva Cardoso

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Sumário

Resumo

O aumento da temperatura do mar é um impacto das mudanças climáticas e o aumento do tempo de submersão das zonas entre-maré pode ser uma consequência direta do aumento do nível o mar. O capítulo único, contido nesta dissertação, foi submetido para o Special Issue da revista Ecological Indicators (SeventIMCO, the 17th International Meiofauna Conference: Meiofauna in a changing world). O objetivo deste artigo é avaliar a resposta de nematódeos de praias arenosas frente ao aumento da temperatura e do tempo de submersão através de um experimento *ex-situ*. A comunidade de nematódeos foi submetida a uma maior temperatura (30°C) e a um período de submersão mais longo (7h) e comparada a um controle (26°C e 4h). O design experimental consistiu em 20 unidades experimentais alocadas em 4 microcosmos: temperatura normal e submersão normal-TNSN, temperatura normal e submersão aumentada-TNSA, temperatura aumentada e submersão normal-TASN, temperatura aumentada e submersão aumentada-TASA. Marés foram simuladas duas vezes ao dia em duas condições: 8h de emersão *vs* 4h de submersão e 5h de emersão *vs* 7h de submersão para os microcosmos de submersão normal e submersão aumentada, respectivamente. Quatro réplicas de cada microcosmo foram removidas no início do experimento (dia 0) e após 15 e 30 dias. Índices taxonômicos e funcionais foram calculados e características e tratos funcionais da comunidade foram analisados. Houve redução de aproximadamente 60% na densidade de nematódeos em D0 comparada ao controle de campo (CC) e a comunidade de CC foi significativamente diferente daquelas observadas nos tratamentos de D0. Houve redução da riqueza genérica, de indivíduos com cutícula estriada e cauda cônica, do c-p 2 e no trato 28, e aumento da abundância de nematódeos com anfídeo circular e dos tratos 21 e 24 no tratamento TASN. Não houve efeito da submersão nas variáveis avaliadas. No tratamento TASA foi observado redução da equitabilidade, aumento da abundância de nematódeos comedores de depósito seletivo e diferentes comunidades de nematódeos entre os tratamentos TNSN e TASA. Esses resultados sugerem que o aumento da temperatura sozinha é suficiente para causar mudanças estruturais e funcionais na comunidade de nematódeos, entretanto, a combinação dos dois fatores pode levar a modificações mais intensas na comunidade. Portanto, o aumento de temperatura causa alterações nas comunidades, mas a soma dos fatores parece ser mais importante do que apenas um único fator isolado.

Palavras-chave: Temperatura; Submersão; Nematoda; Experimento; Microcosmo

Abstract

Increasing sea temperature is one of the climate changes impact and the increase of submersion time of intertidal zones can be a direct consequence of the sea level rising. The unique chapter contained in this dissertation was submitted for the Special Issue of Ecological Indicators journal (SeventIMCO, the 17th International Meiofauna Conference: Meiofauna in a changing world). The aim of this article is to assess the response of sandy beach nematodes to the increase of temperature and submersion time by means of an ex-situ experiment. Nematodes assemblage was submitted to a high temperature (30°C) and a longer submersion period (7h) and compared to a control (26°C and 4h). Experimental design consisted of 20 experimental units placed in 4 microcosms: normal temperature and normal submersion-NTNS, normal temperature and increased submersion-NTIS, increased temperature and normal submersion-ITNS, increased temperature and increased submersion-ITIS. Tides were simulated twice a day in two conditions: 8h of emersion *vs* 4h of submersion and 5h of emersion *vs* 7h of submersion for normal submersion and increased submersion microcosms, respectively. Four replicates of each microcosm were removed in the begging of the experiment (day 0) and after 15 and 30 days post-placement. Taxonomic and functional indices were calculated, and functional characteristics and traits of assemblages were analyzed. There was a reduction around 60% in the nematode density at D0 compared to field control (FC) and the assemblage of FC was significantly different from those observed in D0 treatments. There were a reduction of genera richness, of individuals with striated cuticle and conical tail, of c-p 2 and in trait 28 and increase in the abundance of nematodes with amphid circular and traits 21 and 24 in ITNS treatment. There was no effect of submersion on the variables assessed. In ITIS treatment was observed reduction of equitability, increase in abundance of selective deposit feeder nematodes and different nematode assemblages between NTNS and ITIS treatments. These results suggest that increasing temperature alone is enough to cause structural and functional changes in nematode assemblages; however, the combination of both factors could lead to more intense modifications in assemblage. Therefore, increasing temperature cause assemblages' alterations, but the sum of factors looks to be more important than just one factor isolated.

Keywords: Temperature; Submersion; Nematoda; Experiment; Microcosm.

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Introdução

As mudanças climáticas são um dos perigos mais desafiadores que os ecossistemas estão enfrentando no momento presente e tem causado diversos impactos sobre os ecossistemas marinhos em escala global (Harley *et al.,* 2006; IPCC, 2014). Tais mudanças englobam o aumento da concentração de gases do efeito estufa, principalmente o dióxido de carbono (CO_2) atmosférico, que tem como consequência direta, o aquecimento global (Bindoff *et al.,* 2007), levando ao aumento de temperatura da água do mar, e a acidificação dos oceanos (Doney *et al.,* 2009).

Estes dois impactos – aumento da temperatura e diminuição do pH do oceano, estão entre algumas das mais severas consequências das mudanças climáticas para o ecossistema marinho (Brierley e Kingsford, 2009; IPCC, 2014). O aumento da temperatura, tanto do ar quanto do oceano, leva ao degelo das calotas polares e ao aumento do nível do mar (IPCC, 2013). Além disso, os efeitos das mudanças climáticas, também, podem ser combinados, se considerarmos que qualquer ambiente se encontra constantemente exposto a diferentes tipos de pressões antrópicas, como: despejo constante de poluentes orgânicos e químicos, sobrepesca e perda ou fragmentação de habitats (Turra e Denadai, 2015).

Embora as mudanças climáticas sejam amplamente discutidas no âmbito internacional, o entendimento de como elas afetam o ambiente marinho ficou muito aquém do conhecimento das suas consequências nos ecossistemas terrestres. Isto se deve, provavelmente, ao tamanho e complexidade do oceano, assim como a relativa dificuldade de fazer medições nos habitats marinhos (Hoegh-Guldberg e Bruno, 2010). Como consequência destas características e dificuldades em relação ao ambiente marinho, o estudo sobre o impacto das mudanças climáticas na biota de praias arenosas torna-se complexo, uma vez que este ecossistema é muito dinâmico e diretamente influenciado pelo mar e as alterações que nele ocorrem.

Devido aos fatores físicos (i.e. energia das ondas, marés, ventos, temperatura e chuvas) que controlam o ecossistema de praias arenosas (McLachlan e Brown, 2006), este ecossistema pode, portanto, estar vulnerável aos possíveis impactos das mudanças climáticas, assim como a sua fauna. Dentre tais impactos, destacam-se os fatores físicos que interferem diretamente na fisiografia da praia, como o aumento do nível do mar, na frequência e magnitude de eventos extremos, da taxa de erosão costeira, e as alterações da amplitude das marés, da descarga de sedimentos oriundos de rios e na direção e intensidade das ondas; como, também, fatores que alteram as características químicas do

ambiente marinho ocasionando a elevação da temperatura e a acidificação dos oceanos (Bindoff *et al.,* 2007; Trenberth *et al.,* 2007; McGlone e Vuille, 2012).

Levando em consideração os impactos das mudanças climáticas que afetam os ecossistemas de praias arenosas, certamente os organismos bentônicos habitantes das regiões entre-marés estariam mais suscetíveis a determinados tipos, tais como o aumento da temperatura e do tempo de submersão. À medida que o aquecimento global vem ocorrendo, pode ser que não haja tempo suficiente para que as espécies se adaptem às novas condições ambientais causadas pelas mudanças climáticas em seu habitat natural, principalmente em populações que apresentam baixas taxas de *turnover* (Kelly *et al.,* 2012). O mesmo pode ocorrer em relação ao aumento do tempo de submersão, causado pelo aumento do nível do mar. Nesse caso, haveria, também, a redução ou a perda da zona entre-marés, o qual ocasionaria alterações na riqueza e abundância das espécies, assim como alterações no funcionamento do ecossistema (Yamanaka *et al.,* 2010; Schoelman *et al.,* 2014). Com o encurtamento da região entre-marés, a fauna residente do sedimento de praias arenosas, principalmente aquela com capacidade de dispersão limitada (Celenteno e Defeo, 2016), como é o caso da meiofauna, estaria mais suscetível aos impactos previamente citados.

A meiofauna compreende a comunidade de metazoários mais diversa do ambiente marinho (Giere, 2009), um grupo ecológico heterogêneo, possuindo mais de 25 filos e ocorrendo em diversos ambientes sedimentares (Balsamo *et al.,* 2010; Giere, 2009). Os principais representantes da meiofauna incluem copépodes e nematódeos (Giere, 2009), que são os organismos dominantes e mais diversos do grupo (Pereira *et al.,* 2010).

Estudar os efeitos das mudanças climáticas sobre a meiofauna, e especialmente sobre os nematódeos, pode fornecer informações sobre como os ecossistemas bênticos irão responder aos futuros cenários dos oceanos, uma vez que a meiofauna e nematódeos são bons bioindicadores de mudanças e de qualidade ambiental (Moreno *et al.,* 2011; Balsamo *et al.,* 2012; Zeppilli *et al.,* 2015; Costa *et al.,* 2016). Porém, pouco se sabe sobre como as comunidades da meiofauna irão responder às mudanças climáticas (*e.g.* Danovaro *et al.,* 2001).

Além disso, algumas características dos nematódeos também compartilhadas com outros integrantes da meiofauna, tais como: tamanho diminuto, ciclo de vida curto e ausência de fase planctônica, permitem uma manutenção e manipulação das

comunidades em microcosmos de forma similar aos sistemas naturais em laboratório (Austen, 1989; Olafsson e Elmgren, 1991; Sundelin e Elmgren, 1991). Estas características possibilitaram a manipulação, a partir de experimentos laboratoriais, de comunidades da meiofauna para investigar os efeitos ecológicos (i.e. mudanças em abundância, densidade, composição e riqueza) de variáveis ambientais (Austen, 1989; Olafsson e Elmgren, 1991) e fatores antrópicos nas comunidades como um todo (*e.g.* Warwick *et al.,* 1988; Sundelin e Elmgren, 1991; Austen *et al.,* 1994, 1997; Millward e Grant, 1995; Carman *et al.,* 1995).

Uma série de experimentos que visam buscar explicações para as respostas da meiofauna/nematódeos sob a influência de diferentes impactos causados pelas mudanças climáticas já foram realizados, como, por exemplo, o aumento da temperatura (Gingold *et al.,* 2013; Meadows *et al.,* 2015; Lee *et al.,* 2017; Sarmento *et al.,* 2017; Ingels *et al.,* 2018; Mevenkamp *et al.,* 2018; Vafeiadou *et al.,* 2018a,b), o aumento da incidência de chuvas (Vanaverbeke *et al.,* 2009) e a acidificação dos oceanos (Barry *et al.,* 2004; Meadows *et al.,* 2015; Sarmento *et al.,* 2015, 2017; Ingels *et al.,* 2018; Mevenkamp *et al.,* 2018). Dentre esses, apenas dois avaliaram a assembleia de nematódeos de praias arenosas (Gingold *et al*., 2013; Vafeiadou *et al.,* 2018b).

Em relação à acidificação dos oceanos, sabe-se que a exposição da comunidade infauna de mar profundo ao CO_2 líquido resulta em altas taxas de mortalidade (Barry *et al.* 2004), as quais induzem importantes mudanças na estrutura das comunidades marinhas bênticas associadas a recifes de corais. A maioria dos grupos da meiofauna, presentes nos corais, apresentam respostas divergentes a acidificação (Sarmento *et al.* 2015, 2017), diminuindo a abundância da meiofauna e riqueza de espécies (Lee *et al.,* 2017). Já no caso do aumento da temperatura, mudanças na comunidade de nematódeos de praias arenosas são consequências de hipóteses que relacionam a diversidade específica com o funcionamento do ecossistema, por exemplo, o Modelo de Rivet, o qual prediz que uma comunidade com alta diversidade funciona melhor que uma com baixa diversidade e que a perda da funcionalidade dessa comunidade depende da perda de espécies; e o Modelo da Idiossincrasia, a qual afirma que a resposta funcional depende da perda de espécies e, portanto, não pode ser predita (Gingold *et al.* 2013). Além disso, ainda em relação ao aumento da temperatura, observa-se alteração na dinâmica das espécies pertencentes as comunidades meiofaunais

(Vafaiadou *et al.* 2018b), mudanças no *fitness* de espécies de nematódes de praias arenosas assim como alterações das interações interespecíficas (Vafeiadou *et al.* 2018a). Sobre o aumento da incidência de chuvas, pode haver redução na abundância de nematódeos e mudanças na composição da comunidade, devido a mudanças nos níveis da salinidade intersticial, causando estresse osmótico nos nematódeos (Vanaverbeke *et al.,* 2009). Levando em consideração os efeitos combinados da diminuição do pH oceânico e aumento de temperatura do mar, ocorre a alteração na estrutura da comunidade de nematódeos, que passa a ser dominada por poucas espécies oportunistas (Lee *et al.,* 2017), a alteração da composição do meiobentos, a qual afeta a densidade dos grupos menos dominantes, provavelmente por mudanças nas interações bióticas, enquanto as densidades de nematódeos são menos afetadas (Mevenkamp *et al.,* 2018). Mudanças no pH e temperatura podem, também, afetar substancialmente a estrutura e características funcionais da meiofauna e comunidade de nematódeos (Meadows *et al.,* 2015), sendo as comunidades meiofaunais e de nematódeos mais afetadas em sedimentos lamosos do que em sedimentos arenosos (Ingels *et al.,* 2018).

Nos últimos anos foi ressaltada a importância de abordagens experimentais incluírem múltiplos estresses, já que estes são necessários para revelar as complexas relações ecológicas e biológicas, além de avaliar as respostas a nível de espécie, comunidade e ecossistema a ambiente marinho em mudanças (Zeppilli *et al.,* 2015; Ingels *et al.,* 2018). Apesar do efeito do aumento da temperatura sob a comunidade de nematódeos de praias arenosas já ter sido estudado, o uso da exposição desta comunidade a um maior tempo de submersão como *proxy* para o aumento do nível do mar torna o experimento mais verossímil ao ambiente natural de praias arenosas, como sugerido por Gingold *et al.* (2013). O objetivo deste trabalho é avaliar, por meio de uma abordagem experimental, os efeitos combinados causados pelo aumento de temperatura e tempo de submersão em nematódeos de praias arenosas usando como *proxy* a diversidade taxonômica e funcional desta comunidade. Além de esse ser o primeiro trabalho a testar a simulação de marés, também trazemos abordagens taxonômicas e funcionais, diferindo de outros trabalhos que apenas avaliaram a comunidade de nematódeos baseando-se nos aspectos ecológicos e taxonômicos triviais (densidade e diversidade) e descrevendo a relação entre estes aspectos e os fatores físicos e/ou químicos manipulados nos experimentos.

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Capítulo único

Can the impact of the future climate change affect taxonomic and functional diversity and structure of the sandy beach nematode assemblages? A case study from Fora Beach, Rio de Janeiro, Brazil.

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Abstract

Increasing surface sea temperature is one of the most known climate changes and the increase of submersion time of intertidal zones can be a direct consequence of the sea level rising, which occurs due to the movement of the waterline towards the continent. We aim to assess the response of intertidal sandy beach nematodes due to the increase of temperature and submersion time by means of an *ex-situ* experiment. Midlittoral nematode assemblage was submitted to a higher temperature (30°C) and a longer submersion period (7h) and compared to a control situation (26°C and 4h of submersion). The experimental design consisted of 4 microcosms which four experimental units from each microcosms were removed at days 0, 15 and 30 post-placements. Taxonomic, functional indices, functional characteristics and traits of nematodes were investigated. It was observed a reduction of genera richness and modifications on the abundance of functional characteristics and traits in increased temperature treatment. There was no effect of submersion on variables measured in this work. Meanwhile, a reduction of the equitability, increase in the abundance of selective deposit feeder nematodes and different nematode assemblage occurred when both factors were analyzed in synergism. These results suggest that increasing temperature can cause changes in nematode assemblages and that the synergetic effect could lead to a faster and greater alteration in the assemblage. Therefore, increasing temperature causes assemblages' modifications, but the sum of factor seems to be more important than just one isolated.

Keywords: Temperature; Submersion; Nematoda; Microcosm; Biodiversity.

1. Introduction

Increasing sea surface temperature is one of climate changes consequences as well as one of the most common impacts on coastal marine ecosystems (IPCC, 2007). In intertidal zones, high water temperature associated to long term exposure combined with increased air temperature during low tides could exceed the tolerance limit of some organisms living there and likely causing local extinctions (Brierley and Kingsford, 2009).

Increasing submersion time of intertidal zones is a direct result of sea level rise which consequently leads to the waterline mark to move towards the continent (Defeo et al., 2009). Sea level rising is one of the biggest impacts faced by all coastal ecosystems, including sandy beach ecosystems. In this sense, this ecosystem could exhibit physiography changes, such as reduction or loss of the intertidal region, which will lead to species richness, abundance and ecosystem functioning changes (Yamanaka et al., 2010; Schoelman et al., 2014). A narrow intertidal zone represents a restrict habitat with absence of spatial refuges for sandy beaches benthic fauna with limited mobility (Celenteno and Defeo, 2016). Therefore, benthic organisms inhabiting intertidal zones would be more susceptible to impacts of increasing temperature and submersion time, like meiofaunal organisms.

For many decades sandy beaches were considered marine deserts; however, it is currently known that the sediment acts as substrate to many benthic organisms (Gray, 2002). Among these organisms, we can highlight meiofauna – the most diverse marine metazoan assemblage, mainly composed of nematodes and copepods (Giere, 2009). Free-living marine nematodes are the most abundant meiofauna organisms and may account for up to 80% of total assemblage (Giere, 2009).

Along with their high density on meiofauna samples, nematodes are functionally essential in the sediment. Their role includes the metabolization equivalent to twice the total carbon metabolized by macrofauna in the same area (Heip et al., 1979), oxygenation of the sediment (De Mesel et al., 2003), food supply to high trophic levels, stimulation of bacterial metabolism, which facilitates organic matter remineralization and sediment bioturbation (Moens et al., 2014), improvement of micro-phytobenthic biofilm (Hubas et al., 2013). Nevertheless, its functionality in sandy beaches is still not well comprehended (Esteves and Fonseca-Genevois, 2006).

Functional diversity directly reflects ecosystem processes (Schratzberger et al., 2007) and it is an important biodiversity component. Compared to taxonomic diversity, quantifying functional diversity methods are poorly developed (Petchey and Gaston, 2002). Association stablished between nematode biological characteristics and environmental factors are more informative than those between environmental factors and taxonomic characteristics (Schratzberguer et al., 2007; Armenteros et al., 2009).

There are very few studies demonstrating the functional diversity response of nematodes to some sort of impacts, for instance Vanaverbeke et al. (2004) showed that the enhancement of organic matter deposition in sediment leads to increasing the occurrence of deposit feeders and epistrate feeders nematodes; Gallucci et al. (2015) found that high concentrations of antifouling paint triggers the decreasing of predator nematodes, which majority presents smooth cuticle, suggesting that this cuticle type is more permeable than the ornate ones (Fonseca and Fehlauer-Ale, 2012); Gingold et al. (2013) evidenced that increasing water temperature contributes to predator nematodes loss, which are key role in interstitial trophic webs. On the other hand, there are quite very few studies investigating the impacts caused by climate changes on meiofauna/nematode assemblages. Some authors as Gingold et al. (2013), Meadows et al. (2015), Lee et al. (2017), Sarmento et al. (2017), Ingels et al (2018), Mevenkamp et al. (2018) and Vafeiadou et al. (2018a, b) investigated how temperature increase affect those assemblages; Vanaverbeke et al. (2009) inquired into increase of rainfall incidence; and Barry et al. (2004), Meadows et al. (2015), Sarmento et al. (2015, 2017) Lee et al. (2017), Ingels et al. (2018), Mevenkamp et al. (2018) studied the ocean acidification. Among these studies, only two evaluated sandy-beach nematode assemblages (e.g. Gingold et al., 2013; Vafeiadou et al., 2018b). Gingold et al. (2013) suggested that a more realistic experimental approach should incorporate a longer submersion time as a proxy for sea level rising. Besides to approximate the laboratory condition to natural environmental of sandy beaches other previous studies emphasized that experimental approaches should include multistressor (Zeppilli et al., 2015; Ingels et al., 2018). Multistressors are needed to reveal complex ecological and biological interactions and assess species, community and ecosystem-level responses to a changing marine environment. Aside from being the first paper to expose the meiofauna assemblage to a longer submersion period by simulating a longer tidal regime, this paper also conjugates taxonomic and functional approaches. All the other investigations of the

impact of climate changes on the nematode assemblages are based on trivial ecological aspects (i.e. density and diversity).

The aim of this paper is to understand the synergistic effects of temperature increase and increase of submersion time on sandy-beach nematodes (in relation to taxonomic and functional diversity) by means of an experimental strategy. We have as a hypothesis that the increase of submersion time and temperature together will cause decreasing in taxonomic diversity and changes in functional composition of sandy-beach nematode assemblages.

2. Material and methods

2.1. Study area

Original assemblage for the experiment was collected on Praia de Fora (22°94'S, 43°15'W). This beach is located at Fortaleza de São João, a military area with restricted access in Rio de Janeiro, Brazil. Praia de Fora has 0.4 km of extension and is classified as an exposed beach according to McLachlan (1980) with mean grain size of 350 μm (Cardoso et al., 2016). The mean sea water temperature in this area ranges from 17.75° to 24.18° C in July according to data collected by float systems which monitors sea water surface temperature. This data is available on SiMCosta Portal (www.simcosta.furg.br).

2.2. Sampling original assemblage

A week before the beginning of the experiment, on July 17th 2018, four replicate samples were collected using a 10 cm deep core of 10 cm² in order to provide baseline information concerning the local nematode assemblage (field control). A large volume of sediment was collected from the beach surface (to a depth of 15 cm). Samples for granulometry analysis, total organic matter (TOM) and microphytobenthos (MFB) were also collected as well as 120 L of sea water.

2.3. Experimental set-up

In the laboratory, the sediment was homogenized and used to fill eighty experimental units (EU), which consisted of a plastic beaker (156.25 cm² of surface area) with a small part of the bottom previously removed and covered with a mesh of 38 μm to

enable water flow. Four microcosms were set up consisting of 20 EUs filled with homogenized sediment inhabited by natural meiofaunal sandy-beach assemblages. Macrofaunal organisms were previously removed to the sediment to avoid rapid anoxic conditions in case of death. Natural light (12/12h) and tide regimes (4h of submersion or 8h of emersion) were simulated during the whole experiment. Each microcosm were covered by a thin sea water layer and kept under controlled temperature of 26º C during a week in order to acclimatize. The sea water was previously filtered with 38 μm sieve mesh to remove meiofaunal organisms that could be in suspension. Each of the four microcosms consisted of a combination of two plastic boxes: one (50 L) containing 20 EUs and a subsidiary box of 34 L used to reserve the sea water when simulating tides. One corner of the boxes was reserved for the placement of water pumps used to simulate low and high tides as well as air stones attached to silicone tubes connected to an aerator to oxygenate the sea water, avoiding any disturbance of the sediment inside the EUs (Fig. 1).

In all microcosms, tides were simulated twice a day forming a 10 cm water column above the sediment. The temperature increase was obtained and kept constant in the different treatments by means of thermostats with a heater (50 W). Different temperature treatments were separately placed in different climate chambers. In order to avoid evaporation and consequent salinity variations, plastic film was used to cover all microcosms and the experiment were daily monitored. Increases in salinity were controlled by adding distilled water in the microcosms, maintaining the natural sea water salinity (≈ 35).

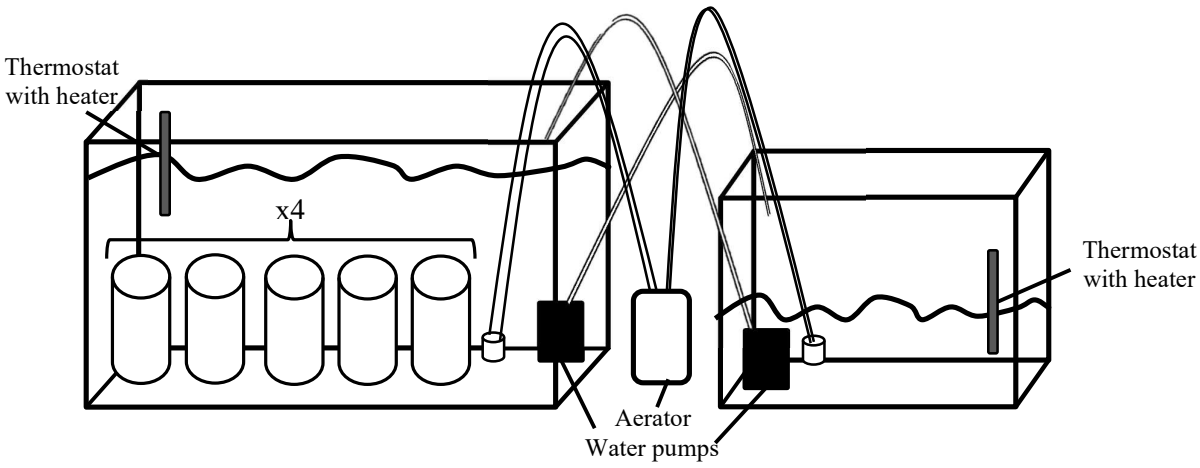

Fig. 1. Schematic drawing of the experimental set up.

To test de effect of increasing submersion time and temperature the eighty EUs were randomly placed at the following treatments - corresponding to the four microcosms previously mentioned:

(1) Normal temperature (26°C) and normal submersion (4h) condition hereafter called NTNS (control)

(2) Normal temperature (26°C) and increased submersion (7h) hereafter called NTIS

(3) Increased temperature (30°C) and normal submersion (4h) hereafter called ITNS

(4) Increased temperature (30°C) and increased submersion (7h) hereafter called ITIS

Four replicates of each treatment were removed during low tide simulation by introducing a 10 cm² core in the EUs. Sediment was collected at days 0 (experiment start), 15 and 30 post-placements. Meiofauna samples were preserved in 4% saline formaldehyde buffered with borax until their analysis. Samples for TOM and MFB were also collected using a 2.37 cm² core. For these samples, an aluminum paper was wrapped to avoid further photosynthesis, and samples were maintained frozen until analysis.

2.4. Samples processing

Nematodes were extracted from the sediment through a combination of decantation and centrifugation with high density solution (Ludox 1.18). Organisms retained in 38 μm mesh sieve were counted using a stereoscopic microscope. A random subsample of 70 nematodes were sorted from each sample and transferred to De Grisse (1969) solution to mount slides for later identification. The identification was done until genera level using the pictorial keys of Warwick et al. (1998) under an optical microscope. All nematodes were picked up when samples had less than 70.

For granulometry and TOM analyses, sediment samples were dried in an oven at 70°C until reach constant weight. After, the sieving methodology was performed for granulometry and loss mass after combustion at 450°C for 4 hours was calculated for the % TOM (Greiser and Faubel, 1988).

MFB analyses were done using active pigments methodology to assess the concentrations of chlorophyll *a* and phaeophytin *a* (Lorenzen, 1970).

2.5. Data analysis

After nematode identification, the following univariate indices were calculated: density (ind./cm²), generic richness (S), equitability (J), Shannon's Diversity (H'), Maturity Index (MI) and Trophic Diversity Index (TDI). MI was calculated as the weighted mean of the individual c-p (colonizer-persistent) values by the formula $MI = \Sigma v(i).f(i)$, where v(i) is the c-p value (1 to 5) of taxon i and f(i) is the frequency of that taxon in a sample (Bongers, 1990). TDI was calculated as $TDI = \Sigma\theta^2$, where θ is the percentage contribution of each feeding type group according to Wieser (1953). TDI values range from 0.25 (highest trophic diversity) to 1.0 (lowest trophic diversity).

Nematodes assemblages of the different treatments and sampling times were analyzed using the following functional characteristics: trophic group (1A: selective deposit-feeders, 2A: epistrate feeders, 1B: non-selective deposit feeders and 2B: omnivores/predators) following Wieser (1953), cuticle type (smooth, striated, punctuated, striated and punctuated, striated with transversal rows), tail shape (short/round, elongated/filiform, conical, clavate), amphid shape (indistinct, slit-like, pocket-like, spiral, rounded or elongated loop, circular, blister-like, longitudinal slit) and life strategy (c-p scale 1 to 5). Altogether 28 categories of biological characteristics were generated from the five functional characteristics exemplified above. A matrix was produced with relative abundance of nematode genera of each sample using these biological characteristics to assess the affinity of each nematode genus to a particular biological trait category.

Permutational analysis of variance (PERMANOVA) was applied to test the differences between the factors treatment (fixed) and time (random) for the univariate (density, S, J, H', MI and TDI) and multivariate (nematodes assemblage and functional characteristics) data using Euclydian distance and Bray-Curtis similarity matrixes, respectively. PERMANOVA was also applied to test the incubation effect on nematode densities and assemblage structure, using only one fixed factor (treatment). A pairwise analysis was performed when significant differences were found to verify where these differences occurred. Homogeneity of data dispersion was tested by a PERMDISP, using the distance among centroids for the factors that showed significant differences in PERMANOVA.

Multidimensional non-metric scaling (nMDS) were applied using Bray-Curtis similarity matrix to observe the nematode assemblage structure and for comparing each

functional characteristic of nematode assemblages to observe possible differences in treatments and times of the experiment. SIMPER analyses were applied to indicate which genus or morpho-functional feature was responsible for the differences in nematode assemblages.

The biological data were analyzed in PRIMER 6 Software with the PERMANOVA package installed (Anderson et al*.,* 2008). Granulometry analyses were performed in Gradistat Software and the sediment was classified according to Wentworth scale (1922).

3. Results

3.1. Environmental data

The granulometric analysis indicated that sediment is composed of medium sand (422.5µm) and moderately well sorted (0.630ϕ) with 0.97% of TOM.

At beginning of the experiment, all treatment showed a TOM lower than the field with the highest amount (0.95%) occurring in the ITNS treatment. At day 15, TOM increased in all treatments, except in ITNS treatment that showed a decrease of TOM. At the end of the experiment (D30), TOM decreased in normal temperature treatments (NTNS and NTIS) and increased in increased temperature treatments (ITNS and ITIS) (Fig. 2A).

Chlorophyll *a* concentration showed a tendency to decreasing during the experiment in all treatments (Fig. 2B). On the other hand, pheophytin *a* did not seem to present any tendency or pattern (Fig. 2C), showing a high value (0.7µg/L) at D0 for NTNS treatment.

3.2. Taxonomic diversity

A total of 16141 nematodes were identified, including 37 genera belonging to 20 families and 7 orders. Thoracostomopsidae was the family with highest number of genera (5) in the experiment, followed by Chromadoridae and Xyalidae, both with 4 genera (Appendix A).

The highest nematode density (1377±40 ind/cm²) was found for FC samples which were significant different from nematode densities found in all treatments at the beginning of the experiment – D0 (Fig. 3A). Along the experiment, nematode densities decreased in all treatments and PERMANOVA result showed significant differences just for the factor time (Table 1).

Generic richness (S) ranged from 13±0.7 to 5.3±1.1 and vary significantly between treatments and time (Table 1), showing a general tendency of increase during the experiment for all treatments, except for ITNS which showed a significant lower value (5.25±1.1) at D30 (Fig. 3B). The highest generic richness (13±0.7) was found for NTNS treatment at D30 (Fig. 3B).

Equitability (J) varied from 0.42±0.02 to 0.81±0.04 and increased in all treatments along the experiment, except for ITIS treatment at D30 which showed a slight decrease. There was a significant difference of J during the experiment (independent of the treatment) and significant lower values were found at D0 (Fig. 3C and Table 1).

Shannon's diversity (H') ranged from 0.29±0.22 to 0.92±0.14, showing a tendency to decrease throughout the experiment. Shannon diversity was significant different among time with higher values being found at the beginning of the experiment (D0) independent of the treatment (Fig. 3D and Table 1).

Nematodes assemblages from FC were significantly different from those from D0 in all treatments (Fig. 4A and Table 1). Comparing only experimental assemblages, there was significant difference between treatment and time (Table 1). NTNS treatment was different from ITIS treatment at D15 and D30. NTIS treatment was also different from ITIS treatment at D30 (Fig. 4B). The highest dissimilarity (49.81%) was found between NTNS and ITNS treatments being five genera (*Microlaimus*, *Theristus*, *Odontophora*, *Comesoma* and *Paracyatholaimoides*) responsible for 70% of the dissimilarity between these treatments (Table 2).

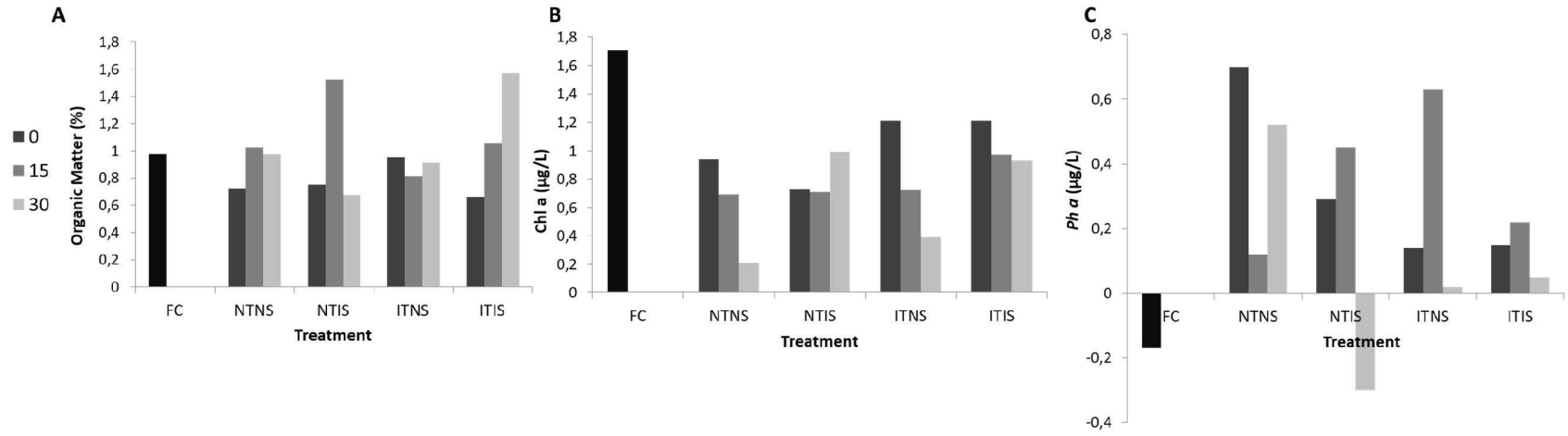

Fig. 2. Environmental data in field control samples and for all treatments along the experiment. A: Organic matter content (%); B: Chlorophyll *a* (µg/L); C: Pheophytin *a* (µg/L) (FC: Field control; NTNS: Normal temperature and normal submersion treatment; NTIS: Normal temperature and increased submersion treatment; ITNS: Increased temperature and normal submersion treatment; ITIS: Increased temperature and increased submersion treatment).

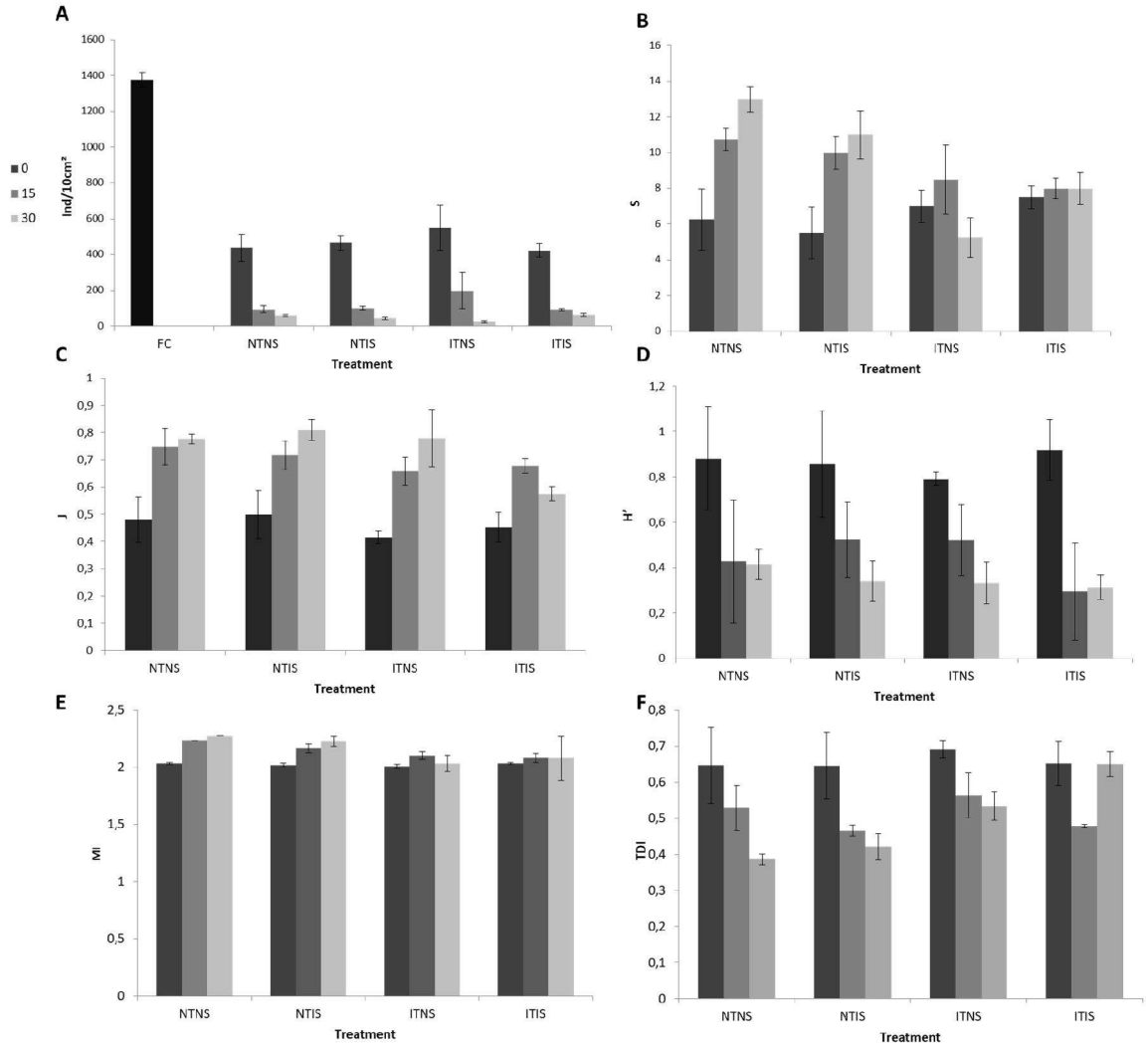

Fig. 3. Biological data in field control and for all treatments along the experiment. A: Nematodes density (ind./10cm²); B: Generic richness (S); C: Equitabily (J); D: Shannon's diversity (H'); E: Maturity Index (MI); F: Trophic diversity index (TDI). Error bar indicates standard error – n: 4 (FC: Field control; NTNS: Normal temperature and normal submersion treatment; NTIS: Normal temperature and increased submersion treatment; ITNS: Increased temperature and normal submersion treatment; ITIS: Increased temperature and increased submersion treatment).

Table 1
PERMANOVA results for taxonomic and functional diversity. Significant values are in bold.

Variables	Treatment			Time			Treatment x Time		
	MS	Pseudo-F	p-value	MS	Pseudo-F	p-value	MS	Pseudo-F	p-value
Density	10805.00000	1.60	0.2721	<0.00001	69.09	**0.0001**	6733.50000	0.57	0.7605
S	21.07600	1.21	0.3929	40.33300	7.59	**0.0023**	17.38900	3.27	**0.0103**
J	<0.00001	2.07	0.1688	0.35416	25.20	**0.0001**	<0.00001	1.02	0.4177
H'	0.58639	2.78	0.1376	2.61100	22.73	**0.0001**	0.21073	1.83	0.1241
Assemblages	2370.50000	1.46	0.2160	34015.00000	37.03	**0.0001**	1623.00000	1.77	**0.0007**
MI	<0.00001	3.45	0.0970	<0.00001	5.05	**0.0086**	<0.00001	0.79	0.6273
TDI	<0.00001	1.20	0.3896	0.12913	10.59	**0.0001**	<0.00001	1.73	0.1453
Trophic group	1761.00000	1.68	0.1803	10462.00000	31.78	**0.0001**	1051.10000	3.19	**0.0001**
Cuticle type	1083.20000	1.52	0.1935	3377.50000	9.98	**0.0001**	710.75000	2.10	**0.0015**
Tail shape	532.88000	0.82	0.6265	2388.80000	11.56	**0.0001**	653.79000	3.16	**0.0001**
Life history (c-p scale)	794.92000	1.13	0.4405	3538.40000	12.85	**0.0001**	701.36000	2.55	**0.0002**
Amphideal fovea shape	1592.90000	1.39	0.2591	7946.60000	21.90	**0.0001**	1149.10000	3.17	**0.0001**
Functional traits	2416.70000	1.69	0.1292	26489.00000	40.98	**0.0001**	1429.30000	2.21	**0.0001**

S: generic richness; J: equitability; H' Shannon's diversity; MI: Maturity Index; TDI: Trophic Diversity Index

Table 2

Genera cumulative contribution to 70% of assemblages' dissimilarity between treatments across all time groups (NTNS: Normal temperature and normal submersion treatment; NTIS: Normal temperature and increased submersion treatment; ITNS: Increased temperature and normal submersion treatment; ITIS: Increased temperature and increased submersion treatment) - : indicate absense of contribuition for the dissimilarity

Genera	NTNS x NTIS (37.95)	NTNS x ITNS (49.81)	NTIS x ITNS (49.50)	NTNS x ITIS (42.59)	NTIS x ITIS (38.41)	ITNS x ITIS (47.68)
Enoploides	59.27	-	-	70.62	-	-
Ingenia	68.05	-	-	-	71.25	-
Paracyatholaimoides	72.34	71.32	69.60	66.33	-	-
Microlaimus	25.78	34.24	34.11	51.09	51.39	38.82
Theristus	42.99	48.49	51.19	29.67	33.86	71.43
Odontophora	54.19	61.44	64.97	61.47	63.01	-
Comesoma	63.75	66.42	73.19	-	67.19	-

3.3. Functional diversity

Maturity Index (MI) values ranged from 2.01±0.01 to 2.27±0.04 and tended to increase during the experiment with significant lower values at beginning of the experiment (D0), except for ITNS and ITIS treatments that showed values similar among the different times tested (Fig. 3E and Table 1).

Trophic diversity index (TDI) varied from 0.39±0.02 to 0.69±0.02 and was significant different during the experiment which higher trophic diversity was found at the beginning of the experiment (D0) independent of the treatment (Fig. 3F and Table 1).

All the four trophic groups of Wieser's were found in all treatments, but there was a significant difference among treatment and time (Table 1). NTNS treatment, with trophic group 2A more representative, was different from ITIS treatment at D15 (1B and 2A were more representatives). At D30, ITIS treatment (1B was more representative) was different from all the other treatments and NTNS treatment (1B and 2A were more representatives) was also different from ITNS treatment (1B was more representative) (Fig. 4C). Highest dissimilarity (31.92%) was found between ITNS and ITIS treatments, being 1B the trophic group contributing for approximately 58% of this dissimilarity (Table 3) and being more abundant (23.5%) in ITIS treatment.

Nematode cuticle, tail shapes, life history and amphid shapes were significantly different between the interaction factor - treatment x time (Table 1). The highest dissimilarities (approximately 28, 22, 26 and 33%, respectively) were found between NTNS and ITNS treatments for all morphofunctional features (Table 3). Striated cuticle, conical tail, c-p 2 with 36, 45, 41% of abundance, respectively, were morphfunctional characteristics more abundant in NTNS treatment. Circular amphideal fovea shape was more abundant in ITNS treatment with 33% of abundance.

Thirty-one functional traits were observed in whole experiment, each trait corresponds to a genus or a group of genera with the same functional characteristics. Functional traits showed significant differences between treatment and time factors (Table 1). At D15, NTNS treatment was significantly different from ITIS and treatments with normal temperature (NTNS and NTIS) were significantly different from ITIS treatment at D30 (Fig. 4H). Highest dissimilarity found for functional traits among treatments was 41.43% comparing NTNS and ITNS treatments, with the combination of traits 21, 28 and

24 corresponding to approximately 53% of 41% dissimilarity between these treatments (Table 3).

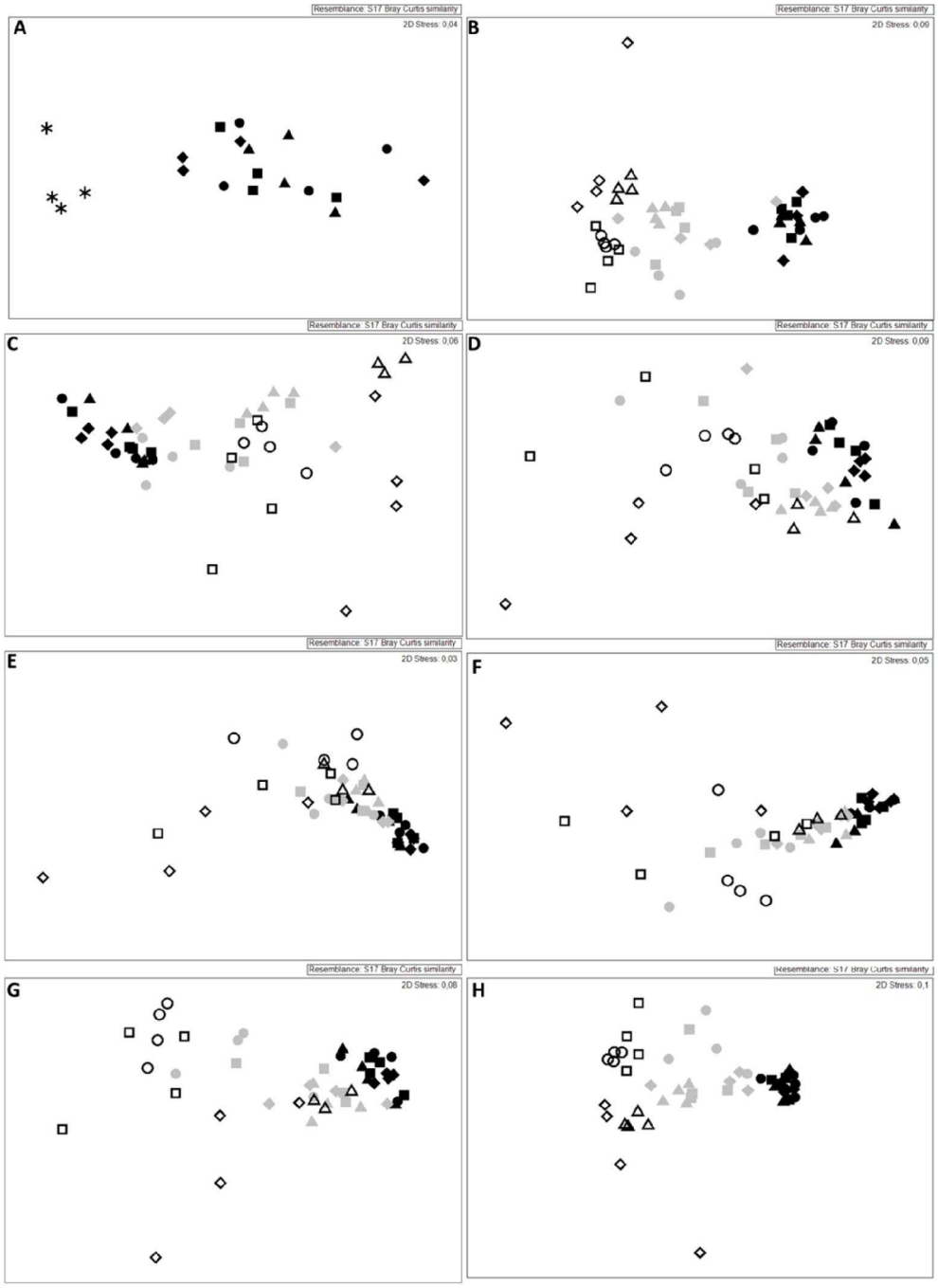

Fig. 4. Nonmetric multidimensional scaling (nMDS) of nematodes assemblage and functional data. A: Experiment incubation assemblages; B: Experiment assemblages; C: Nematodes trophic group; D: Nematodes cuticle type; E: Nematodes tail shape; F: Nematodes life history (c-p scale); G: Nematodes amphid shape; H: Nematodes functional traits. (*: Field control; Symbols in black: Day 0; Symbols in grey: Day 15; Unfilled symbols: Day 30; Circle: NTNS (normal temperature and normal submersion treatment); Square: NTIS (normal temperature and increased submersion treatment); Diamond: ITNS (increased temperature and normal submersion treatment); Triangle: ITIS (increased temperature and increased submersion treatment).

Table 3

Nematodes morphofunctional characteristic dissimilarities and cumulative contribution to 90% assemblages' dissimilarity between treatments across all times group. Highest dissimilarities are indicated in bold (NTNS: Normal temperature and normal submersion treatment; NTIS: Normal temperature and increased submersion treatment; ITNS: Increased temperature and normal submersion treatment; ITIS: Increased temperature and increased submersion treatment; sm: smooth; st: striated; p: punctuated; st/p: striated and punctuated; co: conical; cla: clavate; ; ind: indistinct; pl: pocket-like; spi: spiral; r/el: rounded or elongated loop; cir: circular; - : Indicate absense of contribuition for the dissimilarity).

	NTNS x NTIS	NTNS x ITNS	NTIS x ITNS	NTNS x ITIS	NTIS x ITIS	ITNS x ITIS
Trophic group	18.40	30.51	31.57	26.95	24.96	**31.92**
1B	43.02	80.10	84.13	46.20	52.82	57.69
2A	77.96	51.51	55.02	80.95	84.81	90.27
2B	92.88	95.01	96.82	95.50	96.96	-
Cuticle type	16.66	**28.40**	25.63	20.47	20.73	26.87
sm	48.80	72.19	75.22	72.78	77.72	84.95
st	70.86	54.63	55.82	49.68	56.69	74.40
p	98.40	98.53	-	-	-	93.82
st/p	89.48	89.56	91.66	91.83	91.47	-
Tail shape	10.24	**21.91**	18.31	9.55	9.23	21.51
co	68.39	76.21	84.87	68.20	78.44	85.84
cla	96.87	98.81	98.23	96.89	96.28	99.06
Life history	13.70	**25.85**	21.31	14.87	13.74	23.25
2	63.70	68.59	73.11	59.44	73.35	85.85
3	92.07	93.66	94.74	91.28	93.86	95.60
Amphideal fovea shape	21.19	**33.47**	28.23	30.45	26.42	27.31
ind	74.98	88.55	86.94	79.13	84.12	85.72
pl	96.01	97.06	96.96	-	-	-
spi	89.72	74.65	74.86	90.88	90.52	79.42
r/el	58.33	55.17	60.11	64.31	71.53	91.50
cir	36.57	33.60	33.54	48.57	53.65	69.56
Functional trait	32.56	**41.43**	40.39	39.03	36.06	39.20
Trait 2	88.27	70.33	69.60	77.30	69.89	86.68
Trait 3	55.37	79.30	83.74	69.59	81.47	82.23
Trait 12	73.92	91.64	77.37	-	73.90	88.72
Trait 13	77.69	84.77	80.59	87.38	84.61	-
Trait 14	90.01	89.66	86.08	85.40	89.48	79.83
Trait 15	64.73	75.31	-	73.46	91.02	-
Trait 16	60.11	65.22	63.63	64.95	65.74	90.61
Trait 21	35.58	18.48	21.07	47.59	48.31	67.26
Trait 22	84.37	82.26	90.48	83.20	-	84.49
Trait 24	18.09	53.39	57.46	31.97	34.57	41.92
Trait 26	87.41	87.27	88.33	91.29	87.12	-
Trait 28	49.55	36.16	39.51	60.18	61.49	77.09
Trait 29	69.33	59.66	73.74	80.69	77.82	73.41

4. Discussion

4.1. Experimental set-up

A reduction around 60% in the nematode density at D0 was observed when comparing the density values observed in FC. This result indicates a clear incubation effect before the beginning of the experiment. Similar nematode density reduction was also found by Maria et al. (2011) and according to these authors it could be likely associated to the manipulation of the sediment in sampling, homogenization, transport to the laboratory and setting up of the experimental units. There was also a significant nematode density reduction for experimental treatments during the thirty days of the experiment; this is a very common phenomena for micro- and mesocosms experiments (e.g. Olafsson and Elmgren, 1991; Austen and Warwick, 1995; Schratzberger and Warwick, 1999; Gingold et al., 2013; Vafeiadou et al., 2018b).

It is also important to point out that it was unlikely to have results affected by different cohorts reproducing and overlapping during the experiment since numbers of juveniles (493, 613 and 419 at days 0, 15 and 30, respectively) and adults (369, 164 and 191 at days 0, 15 and 30, respectively) were almost constant along the experiment. Among the nematodes identified, the two dominant genera were *Microlaimus*, in the beginning of the experiment, and *Theristus*, in the end, which seems to substitute the first genus along the experiment. It is known that generation time of a species of *Microlaimus* is approximately 20 days (Tsujino et al., 1997), suggesting two cohorts co-occurring. Several monhysterids, including the family Xyalidae to which the genus *Theristus* belongs, have a generation time of about few weeks to approximately a month depending on the temperature (Chitwood and Murphy, 1964; Hopper and Meyers, 1966; Tietjen, 1967; Gerlach and Schrage, 1971; Heip et al., 1985). Some species of *Theristus*, as *Theristus pertenuis* and *Theristus anoxybioticus*, have dissimilar generation time. *T. pertenuis* had a generation time of 23 days at temperatures between 17°C and 22°C (Gerlach and Schrage, 1971) meanwhile *T. anoxybioticus* had a generation time of one year (Jensen, 1995). Nematode life cycle decreases with increased temperatures, for instance, temperatures between 33°C and 35°C can either interrupt generation time development (i.e. adults fail to deposit eggs, eggs fail to develop or hatched juveniles fail to become mature) or allow adults to survive, but with no guarantee that they are reproducing (Hopper et al., 1973).

Differences in nematode assemblage from FC and those from all experimental treatments at D0 can be attributed to the slight increase of *Microlaimus* abundance and the decrease of *Pseudosteineria* abundance, both occurring at D0 treatments. The density reduction of *Pseudosteineria* could be likely explained by a predator control since there was an increase of the predator *Enoploides* in D0 treatments. Predator control is usually more intense on dominant nematodes (Li et al., 1996) and *Pseudosteineria* was the second dominant genera in FC. On the other hand, the enhancement in the abundance of *Enoploides* may be a consequence of the macrofauna removal, which was done during the experimental set-up to prevent macrofauna death and consequent enhancement of TOM. Albeit the presence of macrofaunal organisms is another factor that become results more realistic, its removal is a common approach adopted for *ex-situ* experiments (e.g. Ingels et al., 2018; Vafeiadou et al. 2018b). When macrofaunal organisms are excluded large nematodes, as the case of *Enoploides* which several species reach from 2 to 7 mm of length (Warwick et al., 1998), are positively affected (Li et al., 1996).

For the experiment, we hypothesized that synergetic effects due to the increase of submersion time and temperature together would cause a decrease in the taxonomic diversity and changes in the functional composition of sandy-beach nematode assemblages. The manipulation of both factors could somehow overcome the physiological optimum of some species, change the behavior and lead less tolerant species to disappear (Bellard et al., 2012). In order to explain those results the discussion section was subdivided into effects of the sole factors (submersion and temperature) and the synergism of these two factors.

4.2. Effects of temperature

When comparing the experimental control (NTNS) with the increased temperature treatment (ITNS), it was observed a reduction of the genera richness, the abundance of nematodes with striated cuticle, the c-p 2 life strategy and the conical tail nematodes as well as trait 28; however, nematodes with circular amphideal fovea shape and traits 21 and 24 increased their abundance.

Genera richness was the unique taxonomic diversity index that reflected changes in the nematode assemblages when there was an increase of 4ºC, contrasting, in part, with results found by Meadows et al. (2015) and Vafeiadou et al. (2018b). Both studies demonstrated that biodiversity by means of taxonomical indexes did not reflect changes in the nematode assemblage under different increased temperature conditions. Meadows et al. (2015) also tested the increase of 4ºC; however, the control temperature tested in their experiment was far lower than the one applied in the current experiment (e.g. 12ºC), besides the effect of decreasing pH was also conjugated to the temperature manipulation. On the other hand, Vafeiadou et al. (2018b) tested increasing temperature in a tropical sandy beach in two different situations: constant increased temperature (+3°C) and fluctuation increasing temperature (+5°C). In this case, it was observed that none of the taxonomic indices evaluated (e.g. abundance, species richness, Shannon-Wiener diversity, Pielou's evenness and Simpson's diversity) were affected by increasing the temperature. But genera reduction found in our experimental situation can agree with the species richness diminution found when temperature was increased from 31 to 36ºC (Gingold et al., 2013).

The highest dissimilarity found between the nematode assemblage from NTNS and ITNS treatments was caused by the occurrence of five genera (*Microlaimus*, *Theristus*, *Odontophora*, *Comesoma* and *Paracyatholaimoides*), which the two first genera were more abundant in ITNS treatment meanwhile the other three genera had a decrease in their average abundance. Along the time of the experiment, it is observed that *Microlaimus* decreased through time, giving space for a more diverse assemblage in NTNS represented by increasing abundances of *Odontophora*, *Comesoma* and *Paracyatholaimus* while in ITNS this genus was substituted by *Theristus*. The substitution of *Microlaimu*s by *Theristus* may be explained due to the reduction of Chl *a* since the first genus is classified as an epistrate feeder (Wieser, 1953) and their preferential food includes microscopic green algae and diatoms. Benthic Chl *a* was measured in the experiment as a proxy for green algae and diatoms that serves as food resource for epistrate feeders. According Bongers (1990), *Theristus* can be classified as a colonizer/opportunistic (r-strategist) nematode showing high tolerance to disturbs and consequently enhances its density/abundance on adverse conditions and could outcompeted with K-strategy genera and benefit from the increased temperature (Ingels et al., 2018). The same life history is attributed for *Microlaimus*, the dominant genus that decreased along the experiment (Bongers 1990), but

as previously mentioned its food supply was depleted limiting its survival. The dominance substitution of *Microlaimus* by *Theristus* might be explained by competitive release, i. e. when decreasing abundance of a dominant genus, as the case of *Microlaimus*, other nematode genera (*Theristus*) are benefited because the available and empty niche can be occupied (Gingold et al., 2013).

Concerning the morpho-functional characteristics, it is difficult to explain the reasons why nematodes with striated cuticle and nematodes with circular amphideal fovea shape are less and more abundant, respectively, when increasing the temperature. Data concerning the relationship of these characteristics and the marine habitat are very scarce (Semprucci et al., 2018). Nevertheless, it is already known that the cuticle can respond to environmental modifications (Bird, 1971), such as habitat hydrodynamics and salinity fluctuations. Sandy beach nematodes are constantly exposed to desiccation and mechanical stress and therefore some type of cuticle, for instance, smooth maybe more sensitive to some sort of impacts (Fonseca and Fehlauer-Ale, 2012) as well as to high concentrations of antifouling paints (Gallucci et al., 2015).

Nematodes with conical tails and cp-2 life strategy also decreased their abundance in ITNS treatment. Tail shapes are important to nematode locomotion, feeding and reproduction (Semprucci et al., 2018). These two characteristics are associated to *Microlaimus* that also decreased its abundance, showing that functional responses are linked to taxonomical results. This achievement indicated that functional analysis reflects results of taxonomic analysis, but when the former is analyzed exclusively is not able to inform which genera are responsible for changes. When applying only the functional analysis some important information can be lost, for instance, the ecological status of an ecosystem which is giving by the presence (relative abundance > 10%) or absence of a specific genera (Moreno et al., 2011).

Decreasing in genus richness and changes on some functional attributes (e.g. striated cuticle, c-p 2, conical tail, circular amphid, traits 21, 24 and 28) partially support our hypothesis that increasing temperature and submersion time acting simultaneously will cause decreasing in taxonomic diversity and changes in functional composition of nematode assemblages, since the manipulation of a single tested factor (i.e. temperature) already caused the expected modifications.

4.3. Effects of submersion

Increasing the submersion periods could lead to some organisms to pass more time submerged and consequently alter their behavior, and those animals which phenotype plasticity could not acclimate to the environmental changes would be extinct and consequently shifts in ecosystem functioning can be caused. Although the effect of the temperature is already evaluated on some experiments (e.g. Gingold et al., 2013; Vafeiadou et al., 2018b), this is the first approach that incorporates tide simulation including the increase of the submersion period. As already postulated the inclusion of this factor on experimental design should incorporate a more trustworthy reproduction of the intertidal environment of sandy beaches (Gingold et al., 2013) because tide is a key environmental variable for this ecosystem.

Even this is the first paper that investigated the possible influence of tide time exposure; there was no evidence on the effect of submersion on the variables investigated in this work.

4.4. Synergetic effects

In the treatment that both analyzed factors were manipulated (temperature and time of submersion), it was observed a reduction of the equitability as well an increase in the abundance of non-selective deposit feeder nematodes besides a complete dissimilar nematode assemblage when comparing it to NTNS and ITIS.

Non-selective deposit feeder nematodes are usually found on fine sediment, due to increase in total organic matter and bacterial growth (Semprucci et al., 2014, 2018) therefore the higher abundance of this feeding type on ITIS treatment may be attributed to the enhancement of TOM which increased 0.91% along the experiment.

In nematode assemblages *Theristus*, at the end of experiment (D30), comprised 67.5% of assemblage abundance on ITIS treatment, largely substituting *Microlaimus*, due to the same reason explained at effects of temperature section (the decrease of Chl *a* because of increasing temperature) which for ITNS treatment the assemblage had 59.8% of its abundance composed by *Theristus* at D30.

In sum, the combined effects of increasing temperature and submersion time seem to cause changes in the nematode assemblage composition which *Theristus* is the dominant genus at the end of experiment. Its dominance reflects the decrease of the equitability in

ITIS treatment at D30. As already presented in the section "effect of temperature", this substitution is given by competitive release and niche availability left (Gingold et al., 2013). But the increment of the dominance of *Theristus* in ITIS may be attributed to the intensification of the impact of one environmental stressor (altered temperature) when other stressors co-occur rather than the interaction of factor (Melatunan et al., 2013). For instance, the reduction of individual species resistance (i.e. *Microlaimus*) to a single stressor seems to occur when exposed to multiple stressors.

5. Conclusion

Increasing temperature change nematodes assemblages' structure, causing genera substitution, but when there is a synergetic effect of increasing temperature and submersion time, those changes occur in a faster way. Functional analysis showed to be important to demonstrate changes in assemblages' structure, although taxonomic effort should be taken in consideration to explain which genera correspond to that change and found reasons for it. With differences in nematode assemblages' structure already occurring at D15 in ITIS treatments, it strongly suggests a greater influence of the synergism of increasing temperature and submersion, although these differences are observed when there only the temperature is manipulated, but in this case this difference is observed fifteen days later (at D30) in ITNS treatment.

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Appendix A

Taxonomic list with biological traits matrix (sm: smooth; st: striated; p: punctuated; st/p: striated and punctuated; st/tr: striated with transversal rows; s/r: short/round; e/f: elongated/filiform; co: conical; cla: clavate; ind: indistinct; sl: slit-like; pl: pocket-like; spi: spiral; r/el: rounded or elongated loop; cir: circular; bl: blister-like; ls: longitudinal slit)

Order	Family	Genera	Functional trait	Trophic group				Cuticle type					Tail shape				Life history (c-p score)					Amphid shape							
				1A	1B	2A	2B	sm	st	p	st/p	st/tr	s/r	e/f	co	cla	1	2	3	4	5	ind	sl	pl	spi	r/el	cir	bl	ls
Enoplida	Enoplidae	*Enoplus*	Trait 1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
	Thoracostomopsidae	*Trileptium*	Trait 2	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0
		Enoploides	Trait 3	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0
		Enoplolaimus	Trait 3	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0
		Epacanthion	Trait 2	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0
		Mesacanthion	Trait 4	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0
	Oncholaimidae	*Oncholaimidae* gen 1	Trait 5	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0
		Oncholaimus	Trait 6	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0
	Enchelidiidae	*Eurystomina*	Trait 7	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
	Ironidae	*Conilia*	Trait 8	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0
		Trissonchulus	Trait 9	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
	Trefusiidae	*Trefusia*	Trait 10	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0
	Lauratonematidae	*Lauratonema*	Trait 11	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0
	Tripyloididae	*Ingenia*	Trait 12	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Chromadorida	Chromadoridae	*Chromadoridae* gen 1	Trait 13	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0
		Endeolophos	Trait 14	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0
		Chromadorita	Trait 14	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0
		Ptycholaimellus	Trait 13	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0
	Cyatholaimidae	*Metacyatholaimus*	Trait 15	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0
		Paracyatholaimoides	Trait 16	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0
	Selachnematidae	*Selachinematidae* gen 1	Trait 17	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
		Synonchiella	Trait 18	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0

Desmodorida	Desmodoridae	*Desmodoridae* gen 1	Trait 19	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0
	Epsilonematidae	*Epsilonema*	Trait 20	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0
	Microlaimidae	*Microlaimus*	Trait 21	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Monhysterida	Siphonolaimidae	*Siphonolaimidae* gen 1	Trait 22	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0
	Xyalidae	*Daptonema*	Trait 23	0	1	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0
		Pseudosteineria	Trait 23	0	1	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0
		Theristus	Trait 24	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0
		Xyala	Trait 25	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0
Aerolaimida	Axonolaimidae	*Apodontium*	Trait 26	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0
		Ascolaimus	Trait 27	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0
		Odontophora	Trait 28	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0
	Comesomatidae	*Comesoma*	Trait 29	0	1	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0
Plectida	Camacolaimidae	*Camacolaimidae* gen 1	Trait 22	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0
		Deontolaimus	Trait 30	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Rhabtidina	Rhabditiidae	*Rhabditis*	Trait 31	1	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0

Conclusões Gerais

- O aumento de temperatura sozinho é suficiente para ocasionar mudanças na diversidade taxonômica, funcional e na estrutura da comunidade de nematódeos.
- O aumento do tempo de submersão não foi possível detectar nenhum efeito sobre a comunidade de nematódeos.
- O efeito combinado do aumento de temperatura e do tempo de submersão também foi capaz de causar mudanças na diversidade taxonômica, funcional e na estrutura da comunidade de nematódeos.
- O efeito combinado é capaz de causar mudanças na estrutura da comunidade mais rapidamente do que apenas o aumento de temperatura.

Anexo: Regras da revista Ecological Indicators.

ECOLOGICAL INDICATORS

Integrating Sciences for Monitoring, Assessment and Management

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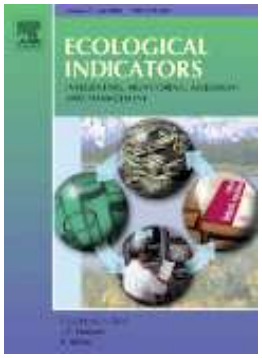

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