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**MICROARTRÓPODES DO SOLO EM UM AGROECOSSISTEMA IRRIGADO COM
ÁGUA DA EXPLORAÇÃO DE PETRÓLEO NO SEMIÁRIDO**

FORTALEZA
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Tese submetida à coordenação do Programa de Pós Graduação em Ecologia e Recursos Naturais da Universidade Federal do Ceará, como parte dos requisitos para obtenção do grau de Doutor em Ecologia e Recursos Naturais. Área de concentração: Ecologia e Recursos Naturais

Orientador: Dr. Olmar Baller Weber

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Aos meus pais que me deram
força e confiança para chegar até
aqui. A minha esposa Wanessa
pelo amor e apoio incondicional.
Sem eles, sem conquistas.

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RESUMO

Em regiões áridas e semiáridas com campos de exploração petrolífera, a água obtida junto com petróleo, conhecida como “água produzida” (AP), vem sendo considerada para irrigação. Devido a importância dos microartrópodes para o funcionamento do solo, foram avaliadas as perturbações promovidas pela AP sobre esses organismos. No capítulo I, foi avaliado se irrigação com AP filtrada e AP tratada por osmose reversa, comparado com água do subsolo captada, altera a estrutura da assembleia de microartrópodes do solo em culturas de mamona e girassol em período seco e chuvoso. A abundância e a riqueza de táxons de microartrópodes foram afetadas pela irrigação e a sazonalidade na mamona. A estrutura da assembleia foi alterada durante a estação chuvosa no girassol, e nas duas estações na mamona. As perturbações promovidas pela AP indicam que a mesofauna do solo pode ser utilizada no monitoramento ambiental da irrigação. No capítulo II, foi avaliado o efeito da irrigação com AP, da AP tratada por osmose reversa e do glutaraldeído, utilizado no tratamento de osmose reversa, sobre os táxons da mesofauna na cultura de abacaxi ornamental. Avaliou-se, através de testes ecotoxicológicos, a reprodução de *Folsomia candida* e *Enchytraeus crypticus* em solo coletado nos tratamentos após um ano de irrigação e em solo contaminado com glutaraldeído. A AP influenciou Hymenoptera, *Cosmochthonius* sp, e Entomobryomorpha. A AP tratada por osmose reversa reduziu a reprodução de *F. candida* e *E. crypticus* e a AP filtrada afetou a reprodução de *E. crypticus*. O glutaraldeído reduziu a reprodução de *F. candida*. Este estudo revela que a AP e o glutaraldeído afetam os táxons da mesofauna reduzindo sua reprodução no solo. No capítulo III as respostas ambientais de ácaros Mesostigmata e do restante dos microartrópodes foram comparadas. Microartrópodes foram coletados em parcelas irrigadas com as três águas e cultivadas com girassol e mamona e na vegetação nativa durante a estação seca e chuvosa. As respostas da abundância e riqueza das duas assembleias em relação a irrigação e sazonalidade foram similares. Os ácaros foram afetados pela sazonalidade no girassol e pela irrigação na mamona e o resto dos microartrópodes por ambos os fatores. Essas assembleias podem ser acessadas para monitorar sazonalidade uso do solo.

Palavras-chave: Ecologia aplicada, Fauna do solo, Monitoramento ambiental, Culturas bioenergéticas, Biologia animal.

ABSTRACT

In arid and semiarid lands with continental oil fields, the water obtained with oil, known as "produced water" (PW), has been suggested as an alternative source for irrigation. Because the relevance of microarthropods assemblage for the soil, in this study was evaluated the disturbances promoted by the PW on those edaphic organisms. In Chapter I, was assessed whether the irrigation with PW filtered and PW filtered and then treated by reverse osmosis, comparing with groundwater from Açú aquifer, changes the mesofauna in sunflower and castor bean crops during dry and rainy seasons. PW and seasonality changed the abundance and richness in castor bean. In the sunflower crop, the assemblage structure was different from the aquifer in PW treated by reverse osmosis during the rainy season, while in castor bean that difference occurred in both seasons. Disturbances promoted by PW on mesofauna can be used for environmental monitoring of irrigation. In Chapter II the effects of PW filtered, PW filtered and then treated by reverse osmosis and the glutaraldehyde, commonly used in treatment of PW by reverse osmosis, on microarthropods taxa was assessed in an ornamental pineapple crop. Ecotoxicological tests evaluated the survival and reproduction of *Folsomia candida* and *Enchytraeus crypticus* on soil samples collected in the treatments after a year of irrigation and in soil contaminated with glutaraldehyde. PW influenced Hymenoptera, *Cosmochthonius* sp. and Entomobryomorpha. The PW treated by reverse osmosis reduced reproduction of *F. candida* and *E. crypticus* and the PW filtered affected the reproduction of *E. crypticus*. Glutaraldehyde reduced the reproduction of *F. candida*. Our study reveals that AP and glutaraldehyde affect the mesofauna taxa reducing their reproduction. In chapter III the environmental responses of total microarthropod and Mesostigmata mites were compared. Microarthropods were assessed in irrigated plots under castor bean and sunflower cultivation, and native vegetation during the dry and rainy seasons. The response of the two assemblages to irrigation and seasonality was similar. Mites were affected by seasonality in the sunflower and by irrigation in castor bean crops, while the total microarthropod have been affected by both factors. Mesostigmata mites or total microarthropod assemblages can be assessed to monitor seasonality and land use.

Keywords: Applied ecology, Soil fauna, Environmental monitoring, Bioenergy crops, Animal biology.

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LISTA DE ABREVIATURAS E SIGLAS

SÍGLA	SIGNIFICADO
AP	Água Produzida
ANOVA	Análise de Variância
NP-MANOVA	Análise Multivariada não Paramétrica
NMDS	Escalonamento Multidimensional não Métrico
pH	Potencial de hidrogênio
GLM	Modelo linear generalizado
CE	Condutividade elétrica
ND	Não detectado
mg	Miligrama
Kg	Quilograma
l	Litro
ds	Decisiemens
m	Metro
mm	Milímetro
BETEX	Benzeno, tolueno, etil-benzeno e xilenos
HPA's	Hidrocarbonetos policíclicos aromáticos
mmolc	Milimol de carga
Na	Sódio
Cu	Cobre
Ca	Cálcio
K	Potássio
P	Fósforo
Fe	Ferro
Mn	Manganês
Zn	Zinco

LISTA DE SÍMBOLOS

SÍMBOLO	SIGNIFICADO
°C	Graus Celsius
≥	Maior igual
≤	Menor igual
=	Igual a
°	Graus
'	Minutos
''	Segundos

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INTRODUÇÃO GERAL

Até a década de 50 do século XX predominava no Brasil a agricultura convencional pouco intensiva e havia conhecimento limitado sobre sinais de degradação do ambiente no meio rural (ALTIERI, 1992). Em décadas posteriores, em razão principalmente do aumento da demanda por alimentos, da agricultura intensiva e da modernização do setor agrícola, grandes áreas de vegetação natural foram transformadas em campos de cultivo. Com a consequente substituição de princípios ecológicos pela especialização na produção, na expansão de monocultivo e na mecanização agrícola, geraram-se também problemas de ordem social, econômico e ambiental (ALTIERI, 1992, 1995). O resultado foi uma intensificação dos processos de degradação do solo em agroecossistemas (MILLENNIUM ECOSYSTEM ASSESSMENT, 2005).

Atualmente, tem-se a degradação do solo como fator de preocupação no setor tecnológico e de produção agrícola. As diferentes formas de agricultura causam mudanças no balanço e no ciclo de nutrientes no ecossistema, limitando suas funções e a autorregulação (GLIESSMAN, 2001). Porém, é na agricultura convencional e intensiva onde se observam paisagens homogêneas e quebra dos ciclos de energia e da matéria orgânica, o que leva a diminuir a biodiversidade do solo (PHILPOTT; ARMBRECHT, 2006).

Dentre as práticas agronômicas que podem interferir na produção de fibras e alimentos e na consequente degradação, citam-se o preparo do solo e a irrigação das culturas (GLIESSMAN, 2001). Em regiões áridas e semiáridas, a irrigação associada à adubação mineral é muitas vezes mal conduzida, resultando em salinização do solo, como o caso do Nordeste do Brasil (CIRILO, 2008; DANTAS; SANTOS; HECK, 1998). No entanto, a irrigação é uma prática de manejo essencial para que haja produção agrícola em ambientes que sofrem de estresse hídrico e não deve ser desconsiderada.

Devido ao problema da escassez de água nas regiões de climas áridos, fontes alternativas de água vem sendo sugeridas para a irrigação, e dentre estas se citam as águas de reuso e as oriundas da exploração de petróleo (BOYSEN, *et al.*, 2002; QADIR, 2003). O uso desse tipo de água pode trazer perturbações adicionais sobre o solo das regiões semiáridas, como a redução da atividade microbiológica (LOPES, *et al.*, 2014), além das que conhecidamente já ocorrem como a salinização

(AL-HADDABI; AHMED, 2007) sendo necessário, portando, o monitoramento ambiental dessa irrigação. Toda água que é retirada junto com o petróleo e o gás de bacias petrolíferas é definida como “água produzida” (aqui mencionada como AP) (LAWRENCE et al., 1995; MACHADO et al., 2006; STEPHENSON, 1992; VEIL et al., 2004).

Na bacia petrolífera potiguar, a exploração de petróleo vem sendo feita desde o final da década de 1970 (MILANI; ARAÚJO, 2003). Segundo a Agência Nacional do Petróleo-ANP, durante os anos em que realizou-se este estudo (2012, 2013 e 2014), foram produzidos aproximadamente 213.147,657 m³ de petróleo e 4.139.864,466 m³ de AP (ANP, 2015). Dessa forma, a produção da bacia potiguar gerou 95% de água e 5% de óleo nesses últimos anos tratando-se, portanto, de poços com baixo rendimento. Toda a AP retirada deve ser devolvida aos poços, visando atender a legislação ambiental vigente (CONSELHO NACIONAL DO MEIO AMBIENTE-CONAMA, 2005).

Em campos com baixo rendimento de óleo, ao invés de devolver a AP ao poços, um uso alternativo dessa água aumentaria a produtividade desses campos (MALONEY; PAETZ, 2002), pois a proporção de água em relação a de petróleo diminuiria ao longo do tempo. A opção de uso da AP passaria pela irrigação de culturas agrônômicas (BOYSEN, *et al.*, 2002; QADIR, 2003) e poderia se transformar em uma fonte de água importante para irrigação em uma região semiárida (ALLEN; ROBINSON, 1993). Tendo isso em vista, foi proposto o uso da AP para a irrigação de culturas não alimentícias, como pastagens e arbóreas (DEJOIA, 2002; JOHNSTON; VANCE; GANJEGUNTE, 2008). A adoção da AP para irrigação em regiões semiáridas traria, portanto, benefícios econômicos tanto aumentando a produtividade dos poços de petróleo, quanto pela renda gerada com a atividade agrícola.

No entanto, têm sido detectados alguns compostos nocivos aos seres vivos na composição da AP tais como: hidrocarbonetos, metais pesados e altas concentrações de sais (ANDRADE et al., 2010; STEPHENSON, 1992). Como a concentração de sais e outras substâncias na AP varia entre as bacias petrolíferas (NEFF, 2002), o seu uso pode ser *in natura*, quando as substâncias nocivas estão em concentrações que as espécies vegetais toleram (OTTON, 1997), ou tratada por osmose reversa, quando as substâncias nocivas como sais encontram-se em um teor mais elevado (NEWELL; CONNOR, 2006). A aplicação ou descarga de AP não tratada e não monitorada pode alterar o padrão de funcionamento de ecossistemas (JANKE;

SCHAMBER; KUNZE, 1992). Como exemplo, elementos minerais, hidrocarbonetos e radionucleotídeos provenientes da AP e acumulados em sedimentos de áreas pantanosas já foram detectados em aves aquáticas (RAMIREZ, 1993; RATTNER et al., 1995). O descarte de AP nessas regiões pode contribuir para a dispersão de substâncias nocivas para outras localidades através de aves migratórias (ESMOIL; ANDERSON, 1995). Ademais, na exploração petrolífera no mar, o descarte pode promover bioacumulação de contaminantes em organismos marinhos, prejudicar a qualidade dos pescados e alterar a cadeia trófica marinha (NEFF, 2002). Até mesmo o tratamento por osmose reversa deve ser avaliado devido ao uso de substâncias biocidas adicionadas à água durante esse processo (MELO et al., 2010).

Com isso, apesar de possíveis benefícios econômicos do uso da AP para irrigação, há de se considerar que tal água possui alto teor de sais e pode conter substâncias tóxicas (NEFF, 2002) e o seu descarte deve ser monitorado. Os compostos dissolvidos poderiam alterar a biodiversidade do solo e comprometer o equilíbrio de processos regulatórios da qualidade do ambiente consistindo, portanto, uma importante fonte de perturbação antrópica.

A fauna do solo pode ser utilizada como indicadora de impactos promovidos pelas práticas agrícolas (BEHAN-PELLETIER, 1999; DINDAL, 1990). As perturbações no solo podem ser observadas através da alteração nas características da população de uma espécie ou na estrutura da comunidade (BERG, 2010; WARD; LARIVIÈRE, 2004). Dessa forma, assembleias de organismos no solo irrigado com AP poderiam sofrer alterações na abundância e riqueza das espécies e, conseqüentemente, na sua estrutura. Tais alterações, quando observadas, podem ser utilizadas para avaliar se a AP promove impactos sobre os organismos do solo.

As perturbações antrópicas e/ou alterações sazonais podem mudar a composição, a abundância das espécies, e a estrutura das comunidades numa escala espaço-temporal (VERHOEF; MORIN, 2010). De uma forma geral, as perturbações ocorrem naturalmente ou podem ocorrer de forma artificial, como a preparação do solo e o plantio, que altera a porosidade natural do ambiente por onde a fauna do solo circula (BATTIGELLI; BERCH, 2002; BRUSSAARD *et al.*, 2007).

A heterogeneidade espacial é um importante fator para a alteração dos padrões de diversidade biológica, dentro de um determinado espaço geográfico, e para determinar a estrutura das comunidades, pois fornece condições para a coexistência de espécies (CHASE; BENGTSSON, 2009; ETTEMA, 2002). Isso se dá

porque a heterogeneidade promove maior diversidade de habitats que, por sua vez, resulta em tipos de recursos diferentes que podem ser disponibilizados às necessidades individuais de espécies distintas (MAGURRAN; MCGILL, 2011).

As perturbações alteram a composição da comunidade ao longo do tempo (BUCKLING et al., 2000) e são representadas por eventos relativamente discretos que removem organismos (TOWNSEND; HILDREW, 1994). Se a perturbação for intensa ao ponto de promover grandes reduções na disponibilidade de recursos, a maioria dos organismos pode não sobreviver e a comunidade em questão tende a um estágio menos diverso (PICKETT; WHITE, 1985). Dessa forma, as espécies mais tolerantes às condições adversas de perturbação podem prevalecer e dominar, alterando assim a estrutura da comunidade (FONG; SMITH; WARTIAN, 2006; PRICE et al., 2011).

Em locais com variações sazonais bruscas de precipitação ou temperatura a diversidade é reduzida, pois esses tipos de perturbações temporais naturais reduzem e aumentam ciclicamente a disponibilidade de recursos e condições (BERG, 2010). O Nordeste do Brasil é uma região onde o recurso que mais varia sazonalmente em disponibilidade é a água. Nesta região, a composição de espécies em áreas onde a disponibilidade de água é baixa pode ser bastante diferente da observada em regiões com água abundante, como nos brejos de altitude (FERNANDES, 1990).

No solo, os fatores que parecem regular os padrões temporais e espaciais referem-se principalmente à heterogeneidade de seu ambiente, especialmente à variabilidade na quantidade e qualidade de recursos e no microclima (BERG, 2010). Espécies vegetais distintas podem fornecer substratos com qualidade química e volumes diferentes (WARDLE, 2002). A importância da disponibilidade e qualidade de recurso (detritos ou resíduos vegetais no solo) para a fauna do solo foi estabelecida a partir da observação que a composição e abundância das espécies são controladas “de baixo para cima” (PIMM, 1982). Sendo assim, flutuações anuais na disponibilização e qualidade de detritos para os organismos do solo podem promover grandes variações sazonais e espaciais nas suas atividades e abundâncias (WARDLE, 2002).

Alterações na qualidade dos recursos podem modificar os padrões das comunidades de organismos do solo (BERG, 2010). Como exemplo, grupos essencialmente microbívoros e detritívoros, como os ácaros oribatídeos, são afetados quando a disponibilidade de fungos diminui (SCHNEIDER; MARAUN, 2005; REMÉN et al., 2010). Nesse trabalho, a nossa premissa foi que a água proveniente da

exploração de petróleo, quando utilizada para irrigação, pode alterar a composição da microbiota (LOPES et al., 2014) e, como consequência, a qualidade dos recursos para a fauna do solo (GRACE LIU et al., 2011; LI et al., 2007) e que essa fauna, nos níveis tróficos acima, depende dos micro-organismos e detritos diversos (WARDLE, 2002; BERG, 2010).

Os invertebrados que habitam e ajudam a decompor o material vegetal depositado no solo são classificados de acordo com as dimensões as quais fazem parte. São estas: microfauna ($\leq 0,1\text{mm}$), mesofauna ($\leq 2\text{mm}$), macrofauna ($\leq 20\text{mm}$) e megafauna ($\geq 20\text{mm}$), sendo a maioria formados por artrópodes (SWIFT; HEAL; ANDERSON, 1979). Claro que essa classificação é categórica e, portanto, os chamados “microartrópodes de solo” ocorrem desde mesofauna até as dimensões menores da macrofauna, sendo que esses organismos possuem capacidade indicadora de perturbação ambiental (O’NEILL et al., 2010). O conhecimento da estrutura das assembleias de organismos do solo serve de subsídio para avaliar o funcionamento do ecossistema solo, fornecendo informações sobre o grau de degradação, modificação ou recuperação de determinada área (DINDAL, 1990).

Uma comunidade pode conter um incontável número de espécies de plantas, bactérias, protozoários, fungos, vertebrados e invertebrados, o que requereria um esforço humano muito grande para inventariar toda sua biota (LAWTON; LARSENK, 1998). Sendo assim, a maioria dos estudos restringem-se a um determinado grupo taxonômico ou a vários táxons identificados em uma categoria taxonômica mais abrangente do que espécies, como os microartrópodes do presente estudo.

Este estudo constou de três capítulos com dados coletados em um agroecossistema no semiárido do Brasil durante os anos de 2012, 2013 e 2014. A estrutura das assembleias de microartrópodes foi avaliada em diferentes ambientes (irrigação com AP x irrigação com outras águas) com uma certa heterogeneidade espacial (sob cultivos espécies vegetais diferentes) e ao longo de uma variação temporal (períodos sazonais distintos). No Capítulo I avaliou-se o impacto da água produzida sobre a estrutura da assembleia de microartrópodes, identificados taxonomicamente no nível de ordem, nas culturas de mamona e girassol. No Capítulo II, foi avaliado como a água produzida poderia afetar individualmente, em campo e em laboratório, cada táxon da mesofauna em solo sob o cultivo de abacaxi ornamental e o efeito da AP e do glutaraldeído, utilizado no tratamento de osmose reversa, sobre

populações de *Folsomia cándida* (Arthropoda: Colembola) e *Enchytraeus crypticus* (Annelida: Oligochaeta). No capítulo III utilizou-se o impacto promovido pela irrigação com AP para medir se as respostas da assembleia de microartrópodes, nas abordagens taxonômicas de Ordem ou Classe, são similares a assembleias de espécies de ácaros Mesostigmata do solo.

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CAPÍTULO I

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IRRIGAÇÃO COM ÁGUA PRODUZIDA ALTERA A ESTRUTURA DA MESOFAUNA EM UM ECOSISTEMA SEMIÁRIDO

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Crisostómo**

Resumo - Devido ao déficit hídrico das regiões semiáridas, fontes alternativas de água para a irrigação devem ser consideradas a fim de melhorar a produção agrícola. O objetivo deste trabalho foi avaliar os efeitos da irrigação com água produzida sobre a estrutura da mesofauna de solo cultivado com mamona e girassol durante as estações de seca e da quadra chuvosa. Foram estabelecidos três tratamentos de irrigação em parcelas cultivadas com mamona e girassol: água produzida tratada por filtração ou tratada por osmose reversa e água do lençol freático. A mesofauna do solo foi determinada durante o período de seco e na estação chuvosa. Apesar da abundância e da riqueza não ser impactada pela água produzida no cultivo de girassol, a estrutura da comunidade foi alterada. Na cultura de mamona, a abundância, a riqueza e a estrutura da comunidade da mesofauna observadas em parcelas irrigadas com água produzida diferiram das que receberam água do tratamento controle. A irrigação com água produzida promove alterações importantes na comunidade o que justifica o uso da mesofauna para monitoramento de agroecossistema irrigados com tal água.

Palavras-chave: Fauna do solo; Estrutura de comunidades; Ácaros do solo; Culturas bioenergéticas; Irrigação com água produzida

PRODUCED WATER IRRIGATION CHANGES THE SOIL MESOFAUNA STRUCTURE IN A SEMI-ARID AGROECOSYSTEM

Abstract - The scarcity of water in semiarid regions requires alternative sources for irrigation to improve agricultural production. Here, we aimed to evaluate the effects of produced water from oil exploration on the structure of soil mesofauna during the dry and rainy seasons in irrigated sunflower and castor bean fields in a Brazilian semiarid region. Three irrigation treatments were applied on plots cultivated with castor beans and sunflowers: produced water treated by filtration (filtrated) or treated by reverse osmosis (reverse osmosis) and groundwater. The mesofauna under the biofuel crops

was collected and identified during the dry and rainy seasons. Although the abundance and richness of the total fauna did not differ between seasons in sunflower plots, the community was altered. In castor beans, the abundance, richness, and community of mesofauna observed in plots irrigated with produced water differed from the groundwater treatment. Irrigation with produced water promotes important changes in soil fauna community that justify their assessment for the maintenance and monitoring of agroecosystems.

Keywords: Soil fauna; Community structure; Soil mites; Biofuel crop; Irrigation with produced water.

1 INTRODUCTION

In semi-arid climates, where the evaporation rate is higher than precipitation, irrigation is used to maintain plant production during periods of inadequate rainfall. However, not all semi-arid regions have high-quality water available for irrigation, so the use of alternative sources, such as wastewater and produced water from industrial oil and gas plants, has been proposed (Allen and Robinson 1993; Johnston et al. 2008). The use of produced water was initially proposed for the irrigation of pastures and tree crops (Dejoia 2002; Johnston et al. 2008). However, the application or discharge of untreated and unmonitored produced water can alter the normal patterns of ecosystem functioning (Janke et al. 1992).

Wastewater and produced water negatively affect the soil and environment, mainly through the amounts of salts that they carry (Cutz-Pool et al. 2007; Köck-Schulmeyer et al. 2011; Tabatabaei and Najafi 2009; Travis et al. 2012). Irrigation with produced water with a high concentration of sodium, chloride, and potassium can cause soil salinization (Al-Haddabi and Ahmed 2007; Melo et al. 2010; Neff 2002). The salinity and sodicity of the soil may reduce the efficiency of carbon use by microorganisms, with consequent changes in their biomass (Ibekwe et al. 2010; Rietz and Haynes 2003).

Reverse osmosis is typically used to reduce produced water salt content. However, the formation of biofilms on membranes requires the addition of a biocide and anti-scalant during the water treatment process (Melo et al. 2010), which may contaminate the water and compromise its quality. Thus, the biocide added to the

reverse osmosis water treatment process could reduce, in the short term, the microbial activity in soil irrigated with that water. (Lopes et al. 2014), which could affect the structure of the soil community.

The soil mesofauna in various ecosystems is influenced by factors such as soil moisture (Frampton et al. 2000; Lindberg et al. 2002; Morón-Ríos et al. 2010; Ukabi et al. 2009; Whitford et al. 1981) and vegetation cover (Bezemer et al. 2010; Ferreira et al. 2012; Franklin et al. 2005). Such factors are related to spatial and seasonal patterns, as well as microclimate variation and the quantity and quality of resources available for soil fauna (Wardle et al. 2006).

In semi-arid regions, soil moisture levels fluctuate between seasons, with accompanying changes in the abundance and composition of soil organisms (Bedano and Ruf 2007; Wallwork 1972; Whitford et al. 1981). Under constant soil water levels, the mesofauna in irrigated agroecosystems is more stable between the dry and rainy seasons, especially in relation to the abundance of organisms, than native forests (MacKay et al. 1986). The low soil moisture level during the dry period has indirect effects on mesofauna through changes in vegetation and reduced activity and diversity of microorganisms in the soil (Acosta-Martínez et al. 2014; Bachar et al. 2010). Thus, seasonal fluctuations in the availability of resources to soil organisms can promote high seasonal variations in the abundance and activity of soil fauna (Wardle 2002).

Although the effects of humidity and vegetation cover on communities of soil fauna are well characterized (Ukabi et al. 2009; Whitford et al. 1981), almost nothing is known about the impact of water quality, especially produced water, on communities of soil fauna. Here, we aimed to evaluate how the quality of water obtained from oil exploration that is used for irrigation can affect the abundance, richness, and community structure of mesofauna in castor bean and sunflower biofuel crops during the dry and rainy seasons in a semi-arid agroecosystem. We hypothesized that irrigation with produced water obtained by simple filtration or treated by osmosis could change the abundance and structure of the soil fauna community in comparison to using groundwater. In addition, we expected that these changes would differ in various crops and seasons.

2 MATERIALS AND METHODS

2.1 Study area

The study was conducted in the experimental area of the farm of the Belém (FZB) oil exploration field managed by Petrobras (4°44'43.2"S, 37°32'19.6"W), located in the Brazilian semi-arid region (see page 78). The soil of the area was classified as Haplic Arenosol and the vegetation as seasonally dry tropical forest, known locally as *caatinga* (Sampaio 1995). The climate is hot and semi-arid in the Köppen classification. The annual rainfall is less than 800 mm and is concentrated from January to May, with an annual mean temperature of 26 to 28°C.

2.2 Experimental design

The study was conducted in irrigated areas cultivated with biofuel sunflower plants (*Helianthus annuus* L. 'BRS 321') or castor beans (*Ricinus communis* L. 'BRS Energia'). The sunflower and castor bean plots were irrigated with three water treatments: groundwater collected from the Açu aquifer (200-m depth); produced water treated by filtration (filtered); and produced water filtered and treated by reverse osmosis (reverse osmosis), all captured in FZB (see page 82). Treatments with water management were composed of three replicates of 400 m² for each crop and were randomly distributed in an area of 19,200 m² (see page 79). Both plants were planted in two cropping cycles in the dry season (September 2012) and rainy season (March 2013, Figure 1.1). The soil mesofauna was collected and identified in all areas in both seasons.

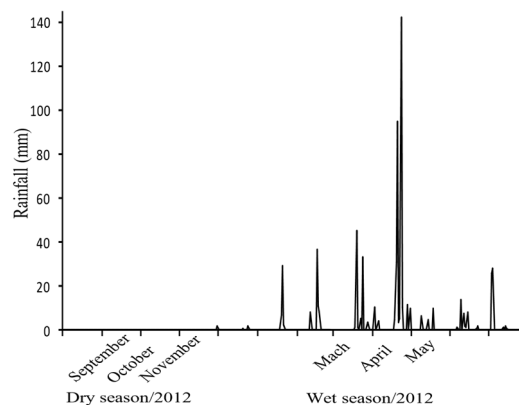


Figure 1.1. Rainfall data collected between August 2012 and July 2013 by the meteorological station installed in the experimental area.

2.3 Irrigation management

A drip irrigation system was used to avoid water contamination among treatments (see page 81). The system was automated and the daily amount of water supplied was estimated based on crop evapotranspiration and soil drainage, measured using a lysimeter column in the plots. Lysimeters were made by technicians of the Brazilian Agricultural Research Corporation (Embrapa, Ceará/Brazil) using plastic columns (0.4 m diameter and 0.7 m deep) where the water drained daily was used to estimate the evapotranspiration of crops. The produced water for the filtered and reverse osmosis treatments had been previously separated in the industrial oil exploration plant (Melo et al. 2010). The water ion concentrations, electrical conductivities, and pH are shown in Table 1.1.

Table 1.1. Chemical composition of water used for the irrigation of castor beans and sunflowers, supplied by the Belém farm.

Waters/season	CE (dS/m)	pH	Ca	Mg	Na	K	Cl
				mmol/l			
Dry							
Filtrated	2.51	8.84	0.11	0.65	24.15	0.68	13.74
Reverse osmosis	0.62	7.35	0.01	0.03	3.75	0.11	2.89
Groundwater	0.65	8.24	0.21	0.10	7.10	0.09	2.06
Rainy							
Filtrated	1.95	9.21	0.11	0.16	18.15	0.56	12.70
Reverse osmosis	0.38	7.52	0.11	0.07	2.95	0.05	1.21
Groundwater	0.66	8.34	0.21	0.11	6.23	0.08	2.41

2.4 Sampling and identification of soil mesofauna

Undisturbed soil sub-samples were collected using cylindrical soil samplers (10-cm diameter; 10-cm depth. See page 80). The sub-samples were placed individually in plastic bags, stored in boxes, and immediately transported to a Berlese–Tullgren apparatus. Nine soil sub-samples were collected in each 400-m² plot at different plant developmental stages (three samples at germination, three at flowering, and three immediately before the harvests, and all were 8 m distant from each other) forming a composite sample for each plot (Table 1.2). The soil mesofauna were extracted using the Berlese–Tullgren method modified by Franklin and Morais, (2006)

and classified at the level of order or sub-order. Holometabolic taxa were further separated into immature and adult organisms.

Table 1.2. Number of soil samples and sub-samples collected in each irrigation treatment for mesofauna extraction in a Berlese–Tullgren apparatus.

Samples/sub-samples	Sunflower	Castor bean	Total
Plots (composite samples) in each season	3	3	6
Sub-samples in each plot in each season	9	9	16
Sub-samples in dry season	27	27	54
Sub-samples in rainy season	27	27	54

2.5 Data analysis

Analysis of variance (ANOVA) was used to evaluate the influence of irrigation treatments and season on mesofauna abundance and the number of taxa. The abundance data were normalized using a $\log x + 1$ transformation. The water treatment means were compared by the Tukey post hoc test, at 5% probability. The sunflower and castor bean irrigation treatments were used to evaluate the effect of water on mesofauna community structure. The Bray–Curtis index was used to generate a similarity matrix among samples. Each matrix was subjected to a Multivariate Nonparametric Analysis of Variance (NP-MANOVA).

To generate a graphical representation of the composition/abundance of soil mesofauna across irrigation treatments, we used Nonmetric Multidimensional Scaling (NMDS) ordered in two dimensions and calculated from the Bray–Curtis index. The scores resulting from the NMDS were plotted, and groupings of samples were evaluated. The statistical program R (R Development Core Team 2014) was used for all analyses.

3 RESULTS

We identified 3022 individuals distributed among 23 arthropod taxa in the studied area. Notably, we did not detect Oribatida (see page 83), Thysanura, Symphyta, or Isopoda in the cultivated and irrigated areas. Some groups, such as Homoptera, Neuroptera larvae, and Poduromorpha were identified only during the dry

season crops, while Blattodea and Orthoptera were found only during the rainy season (Table 1.3).

Table 1.3. Relative abundance (Ab) and relative frequency (Fr) of soil organisms collected from nine castor bean and nine sunflower plots in the dry and rainy seasons.

Taxa	Dry season				Rainy season			
	sunflower		castor bean		sunflower		castor bean	
	Ab	Fr	Ab	Fr	Ab	Fr	Ab	Fr
Astigmata	19.7	100.0	25.2	100.0	12.8	66.7	21.8	100.0
Prostigmata	10.3	44.4	13.7	100.0	9.0	44.4	33.7	100.0
Mesostigmata	5.6	22.2	7.2	33.3	13.6	77.8	14.9	44.4
Acari	35.6	100.0	46.1	100.0	35.4	100.0	70.4	100.0
Aranae			1.6	44.4	5.2	77.8	1.2	100.0
Blatoidea							0.1	11.1
Homoptera	3.5	33.3	0.4	11.1				
Coleoptera (larvae)	24.6	88.9	8.0	88.9	2.3	66.7	2.6	77.8
Coleoptera	18.0	88.9	11.5	100.0	3.8	55.6	0.5	44.4
Dermaptera	0.4	11.1	0.2	11.1			0.1	11.1
Diplura	0.4	11.1			1.5	44.4	0.5	55.6
Diptera (larvae)	1.8	44.4	3.2	44.4	5.2	44.4	0.7	77.8
Entomobryomorpha	7.4	55.6	8.7	88.9	14.2	66.7	6.9	88.9
Formicidae	2.8	44.4	10.7	77.8	9.8	66.7	2.6	77.8
Hemiptera	0.4	11.1	0.4	22.2			0.1	22.2
Hymenoptera (whithout formicidae)			0.2	11.1				
Isoptera	1.1	22.2	5.8	33.3				
Lepidoptera			0.2	11.1				
Lepidoptera (larvae)	0.4	11.1	0.2	11.1			0.1	11.1
Neuroptera (larvae)	0.4	11.1						
Orthoptera							0.1	11.1
Poduromorpha			0.2	11.1				
Pseudoscorpiones					0.4	11.1	0.1	11.1
Psocoptera	0.7	22.2			0.6	33.3	10.8	77.8
Symphyleona	0.7	11.1			20.7	66.7	3.3	77.8
Thysanoptera	2.1	44.4	2.6	44.4	0.8	44.4	0.1	22.2

The average number of organisms collected in sunflower plots was not significantly different between seasons (Table 1.4). Among irrigation treatments, abundance in the groundwater did not differ significantly from the other irrigation treatments. There were no differences in the richness of organisms in sunflower plots between seasons or between plots treated with groundwater and filtrated or reverse osmosis water (Table 1.4).

Table 1.4. Summary of ANOVA testing the effects of season and irrigation treatments on the log-transformed abundance and richness of soil organisms in sunflower and castor bean cultivation.

Variables	Df	Abundance			Richness		
		<i>F</i>	<i>p</i>	Mean (Sq)	<i>F</i>	<i>p</i>	Mean (Sq)
Sunflower							
Season	1	2.035	0.179	24.110	0.645	0.438	2.722
Irrigation	2	0.921	0.424	10.920	0.645	0.542	2.722
Season x Irrigation	2	1.350	0.296	16.000	0.645	0.543	2.721
Residuals	12			11.850			4.222
Castor Bean							
Season	1	12.005	0.005	83.720	6.429	0.026	6.006
Irrigation	2	11.737	0.002	81.850	11.514	0.002	10.757
Season x Irrigation	2	4.574	0.033	31.900	0.086	0.918	0.080
Residuals	12			6.970			0.934

The numbers in bold indicate significant effects.

In castor bean plots, the abundance of organisms collected was significantly different between the seasons. In addition, the irrigation treatments influenced the abundance of soil organisms and there was an interaction with season (Table 1.4). In plots irrigated with groundwater, the abundance of organisms in the dry season differed from other irrigation treatments. In the rainy season, the abundance of organisms in soil receiving groundwater was similar to that in the other plots; however, the reverse osmosis treatment differed from filtered water (Table 1.5). The richness of soil mesofauna in castor bean plots was different between seasons and water treatments (Table 1.4). The number of taxa in groundwater treatments was lower in the dry season than the rainy season. In the dry season, the richness of taxa was significantly higher in the groundwater treatment than the filtrated and reverse osmosis treatments. In the rainy season, the richness was not different from the reverse osmosis or filtered treatments (Table 1.5).

Table 1.5. Tukey post hoc tests comparing the means of soil fauna among groundwater, reverse osmosis, and filtered treatments in castor bean cultivation. Means followed by the same uppercase letter in rows and lowercase in columns did not have significant ($P < 0.05$) pair-wise differences among treatments.

Treatments	Abundance		Richness	
	Dry season	Rainy season	Dry season	Rainy season
Groundwater	17.360 Aa	17.610 Aab	6.470 Aa	7.390 Ba
Reverse osmosis	8.630 Ab	12.000 Ab	3.680 Ab	4.850 Aa
Filtered	10.780 Ab	20.100 Ba	4.620 Ab	6.010 Aa

The composition of soil fauna communities in sunflower plots differed significantly between irrigation treatments, but depended on the season (Table 1.6). The NMDS analysis shows that the differences in mesofauna communities were primarily between reverse osmosis and the other treatments in the rainy season (Figure 1.2). In castor bean plots, the community composition of soil fauna differed significantly between irrigation treatments and between seasons, with no significant interaction (Table 1.6). The plots that were irrigated with filtrated water and groundwater had similar mesofauna community structures in both cropping cycles. Treatment with reverse osmosis water resulted in distinct communities that further differed between seasons (Figure 1.2).

Table 1.6. Summary of non-parametric multivariate analysis of variance (NP-MANOVA) based on 1000 permutations among the mesofauna structure from the Bray–Curtis index with seasonal and irrigation treatments on crops of sunflowers and castor beans.

Variables	Df	F_{model}	R^2	p
Sunflower				
Season	1	6.009	0.217	0.001
Irrigation	2	2.483	0.179	0.006
Irrigation x Season	2	2.346	0.170	0.017
Residuals	12		0.434	
Castor beans				
Season	1	10.531	0.340	0.001
Irrigation	2	2.631	0.170	0.008
Irrigation x Season	2	1.599	0.103	0.115
Residuals	12		0.387	

The numbers in bold indicate significant effects.

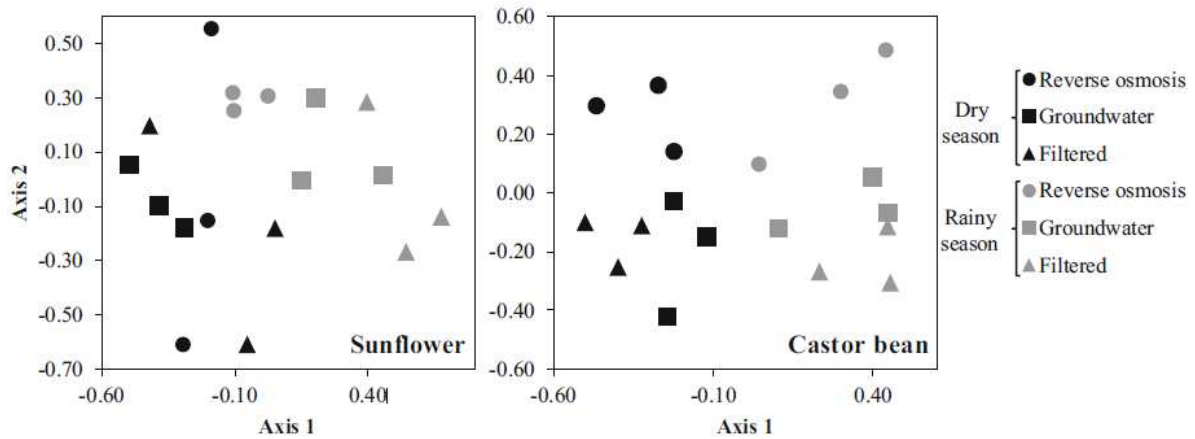


Figure 1.2. Ordination of soil mesofauna in two-dimensional NMS based on Bray–Curtis for sunflower (stress 0.19) and castor bean (stress 0.15) plots. Black points represent the dry season and gray points the rainy season. The triangles represent samples collected in soil irrigated with filtered produced water, the squares represent groundwater, and the circles represent produced water treated by reverse osmosis.

4 DISCUSSION

Here, we presented data on changes in soil mesofauna promoted by irrigation with produced water. The water composition can potentially induce chemical and/or physical changes in the soil, modifying microorganism communities (Ibekwe et al. 2010) and the growth and health of plants (Qadir et al. 2003). Although irrigation with produced water could promote soil salinization (Al-Haddabi and Ahmed 2007; Melo et al. 2010), in our study results, it changed the abundance of organisms in the soil cropped with castor bean during the dry season. Furthermore, changes in soil fauna abundance or richness were not evident between the produced water and groundwater treatments during either season in sunflower plots.

Salinity is known to affect some mesofauna groups, as observed for mites in semi-arid regions of Australia, where there are species differences between high and low salinity soils (Noble et al. 1996). Organisms such as mites are affected by the number and size of soil pores (Nielsen et al. 2008). Filtered produced water contains high Na content and may reduce soil porosity (Al-Haddabi and Ahmed 2007). Soils with high salinity affect populations of microorganisms that are less diverse and less-efficient at carbon source utilization (Ibekwe et al. 2010; Rietz and Haynes 2003). During the rainy season, the accumulated salts from filtered produced water in the soil may be leached, removing any effects on the structure of mesofauna.

Lopes et al. (2014), studying the short-term effects of produced water on microorganism activity, observed larger populations of filamentous fungi when soils were irrigated with filtered produced water and groundwater than with water treated with reverse osmosis in this same study area. Mesofauna groups that are essentially scavengers and microbivores increase their abundance when there is greater availability of fungi in the soil, which subsequently affects higher trophic levels (de Ruiter et al. 1995; Remén et al. 2010; Schneider and Maraun 2005).

In the produced water treatment process, the addition of glutaraldehyde, which is toxic for some organisms (Leung 2001), during the desalinization process of reverse osmosis (Melo et al. 2010) could decrease the microbial activity of the soil (Lopes et al. 2014). On our plots, we observed changes in the abundance, richness, and structure of the mesofauna. Micro-organisms are an important factor on mesofauna in soil food webs (Berg et al. 2001; de Ruiter et al. 1995). It is believed that these factors have an indirect effect on the composition and abundance of soil mesofauna.

Two possible explanations for the differences in the structure of the soil community between different cropping cycles are as follows: (1) salts and other chemicals have accumulated in the soil during the second crop cycle, and (2) the effect of the rainy season in the semi-arid region. The increase in the concentration of salts in the soil (Elkins and Whitford 1984; Noble et al. 1996), as well as biocides such as glutaraldehyde (Leung 2001), during the dry season may have an effect (directly or indirectly) on some mesofauna organisms. This effect may be due to changes in the physicochemical characteristics of the soil microfauna or changes to the food web (Bezemer et al. 2010; de Ruiter et al. 1994; Rietz and Haynes 2003), based on the principle that changes in resource quality may modify the patterns of communities of soil organisms (Berg 2010).

Although there was no discrepancy in the seasonal availability of water in the cultivated irrigated area, we found that the mesofauna changed between seasons. Therefore, we propose that the mesofauna organisms present in the native forest and around the experimental area may have served as a source for the dispersal of organisms to more cultivated soils during the rainy season (Ettema and Wardle 2002; Pulliam 1988). It is noted that many soil fauna organisms can enter a state of quiescence or cryptobiosis during the dry period, or tend to hatch eggs only in the rainy season, which results in their apparent absence during the dry season (MacKay et al.

1987). In addition, fungi that serve as food for many animals are inactive during the dry season (Whitford 1988).

Oribatid mites were absent from the fields of both types of crops. In fact, they are sensitive to soil management in semi-arid environments (Bosch-Serra et al. 2014). Changes in the composition of mesofauna in agroecosystems can be associated with the type of soil and crop management (Bedano et al. 2006; Crossley et al. 1992; Domínguez et al. 2013; Lalley et al. 2006). Compared to native forests, cultivation potentially alters food webs and microhabitats that reduce the diversity and abundance of some groups of soil organisms (Bedano et al. 2006; de Ruiter et al. 1994; Wardle et al. 1995).

5 CONCLUSIONS

Irrigation with produced water promotes important changes in soil fauna structure that justify its assessment for the maintenance and monitoring of agroecosystems. To our knowledge, this is the first evidence of how the mesofauna structure is influenced by the quality of water used for irrigation. In semi-arid regions, seasonal effects naturally induce variations in the composition and abundance of soil organisms, even if the areas are irrigated. The responses of soil fauna to differences in season differ between the land-use types.

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CAPÍTULO II

(Publicado no periódico *Bulletin of Environmental Contamination and Toxicology*)

EFEITO DA IRRIGAÇÃO COM ÁGUA PRODUZIDA ORIUNDA DA EXPLORAÇÃO DE PETRÓLEO SOBRE A MESOFAUNA DO SOLO

Raimundo Nonato Costa Ferreira, Olmar Baller Weber, Maria Elizabeth Fernandes Correia, Eloísa dos Santos Benazzi-Ikeda, Rafael Nogueira Scoriza, Antonio Lindemberg Martins Mesquita

Resumo - A água oriunda da exploração petrolífera pode conter substâncias que alteram a diversidade de organismos do solo. Neste trabalho foi avaliado se a água produzida tratada por filtração, por osmose reversa e o glutaraldeído utilizado no tratamento por reversa afeta negativamente a mesofauna do solo em uma área irrigada. Em campo a irrigação com água produzida tratada por osmose reversa e filtração influenciou Hymenoptera e *Cosmochthonius* sp. enquanto colêmbolos Entomobryomorpha foram afetados pela água tratada por osmose reversa. Nos testes ecotoxicológicos a reprodução de *Folsomia candida* foi influenciada pelo tratamento de osmose reversa e *Enchytraeus crypticus* foi afetado pelos dois tratamentos de água produzida. Apesar do glutaraldeído não afetar a sobrevivência de *F. candida* a reprodução foi inibida (EC50 de 44,43mg/L). Não foi observado efeito do glutaraldeído sobre a reprodução e sobrevivência de *E. crypticus*. Esses resultados levam a sugerir que alguns tipos de água produzida, quando utilizadas na agricultura, podem afetar a mesofauna do solo.

Palavras chave: Reuso de água, Fauna do solo, Toxicologia, Glutaraldeído

EFFECTS OF WASTEWATER FROM OIL EXPLORATION ON SOIL MESOFAUNA

Abstract- Wastewater from oil exploration may contain substances that can alter the diversity of soil organisms. This study evaluated whether produced water treated by filtration or reverse osmosis and glutaraldehyde from reverse osmosis treatments negatively affected the mesofauna in an irrigated area. In the field, irrigation with produced water treated by reverse osmosis and filtration influenced Hymenoptera and *Cosmochthonius* sp., while Entomobryomorpha springtails were affected only by the reverse osmosis water. In the ecotoxicological tests, reproduction in the springtail *Folsomia candida* was inhibited by the reverse osmosis treatment, while reproduction

in the earthworm *Enchytraeus crypticus* was affected by both water treatments. Although glutaraldehyde did not affect the survival of *F. candida*, the reproduction was inhibited (EC50 = 44.4 mg/L). No adverse effect of glutaraldehyde was observed on reproduction or survival of *E. crypticus*. These results indicate that produced water, when used in irrigated agriculture, may affect soil functional mesofauna.

Keywords: Water reuse; Soil fauna; Toxicology; Glutaraldehyde.

1 INTRODUCTION

Treated water from petroleum exploration (produced water) is unfit for human consumption, but can be used for irrigation (Johnston et al 2008). In arid and semi-arid regions with scarce water sources, it is important to reuse water (Travis et al 2012). To be usable in crop irrigation, produced water must pass through treatment processes that remove salts, metals, and other components (Murray-Gulde et al 2003; Melo et al 2010).

Simple filtration of the produced water is not effective in desalting, causing salinization of the soil (Al-Haddabi and Ahmed 2007). Soil salinity can negatively affect the mesofauna (Elkins and Whitford 1984) and also change the microorganism community (Ibekwe et al 2010), which is important as a food resource for soil fauna (de Ruyter et al 1994). Other water treatment processes, such as reverse osmosis and ultrafiltration, are efficient in removing salts and metals. However, in these treatments, glutaraldehyde is used to prevent the formation of a biofilm on the reverse osmosis membrane. This prolongs its service life (Melo et al. 2010), but results in reduced microorganism activity in soil when this water is used in irrigation (Lopes et al 2014).

Any negative impact on soil communities and trophic webs reduces the benefits of these organisms to the soil (Brussaard et al 2007; Barrios 2007). In such cases, mesofauna taxa, such as nematodes, mites, worms, isopods, enchytraeids, and springtails, can serve as biomarkers of pollution (Cortet et al 1999). Because of the importance of these animals in the nutrient cycling processes and their role in the maintenance of the physical and chemical quality of soil (Dindal 1990), the impacts on communities and populations of soil organisms should be taken into consideration when using produced water for irrigation. Because glutaraldehyde is used in disinfecting hospitals, effects have been evaluated only on aquatic organisms such as

mollusks (SINTEF 1991), algae (Sano et al 2005), fishes (Pereira et al 2014), worms, and crustaceans (Sano et al 2003, 2004, 2005, Boillot and Perrodin 2008). However, the direct and indirect effects of glutaraldehyde on soil mesofauna remain unknown. Thus, we hypothesized that the mesofauna will be affected by saline produced water or the glutaraldehyde used in reverse osmosis treatments.

The aim of this study was to evaluate whether irrigation with treated produced water affected soil mesofauna taxa in an agricultural area as compared with groundwater irrigation. We investigated the effect of irrigation on higher-level taxa that are commonly used in environmental monitoring, and on oribatid mite species. This study also evaluated whether the soils under the irrigation treatments or exposed to glutaraldehyde had a potential ecotoxicological effect on the mesofauna species *Folsomia candida* Willem, 1902 (Collembola) and *Enchytraeus crypticus* Westheide & Graefe, 1992 (Oligochaeta).

2 MATERIAL AND METHODS

The study was conducted in fields irrigated with different types of water or non-irrigated land cultivated with ornamental pineapple plants (*Ananas comosus* var. *erectifolius* (L.B. Smith) Coppens & Leal) on the Belém farm (FZB) of Petrobras, located in the municipality of Aracati, State of Ceará (Brazil) (4°44'43.2"S, 37°32'19.6"W). The soil of the area was classified as Haplic Arenosol with the following size fractions (g/kg): 978 sand, 27.4 silt, and 26.6 clay. In this area, air temperature ranges from 26 to 28°C, annual rainfall is up to 800 mm, and it has a hot, semi-arid climate according to the Köppen classification.

The sampling units comprised nine plots of 400 m² that were arranged in a completely randomized design with three replications for each irrigation treatment and distributed in an area of 19,200 m². The three treatments included: 1) control treatment with groundwater (Groundwater); 2) produced water filtered in sand filters and then treated by reverse osmosis (APO); and 3) produced water that was only filtered (APF). The concentration of glutaraldehyde present in the water from the reverse osmosis treatment was approximately 0.198 mg/L (Melo et al 2010). Each treatment modified the chemical characteristics of the soil (Table 2.1).

Tabela 2.1. Soil analysis data from plots irrigated with the three types of water used in this study before and after one year of irrigation.

Soil analysis	Groundwater		APO		APF	
	Before	After	Before	After	Before	After
Electrical conductivity (dS/m ²)	0.57	1.97	0.69	1.87	1.39	2.85
pH (in water 1:2.5)	8.49	8.17	8.08	6.63	8.59	8.07
Ca (mmolc/kg)	10.4	16.7	14.5	18.2	11.1	15.9
Mg (mmolc/kg)	1.98	10.4	2.31	10.2	2.69	9.39
K (mmolc/kg)	0.55	1.74	0.64	1.66	0.54	2.06
Na (mmolc/kg)	0.12	7.45	0.16	4.69	0.15	11.1
Cu (mg/kg)	0.03	1.13	0.03	0.39	0.03	0.16
Fe (mg/kg)	7.13	47.1	6.02	37.2	8.22	35.7
Mn (mg/kg)	14.8	31.2	11.8	30.2	21.56	20.9
Zn (mg/kg)	0.83	5.86	0.91	3.59	0.74	5.96
P (mg/kg)	201	127	286	149	242	141
PAHs ^a	ND ^c	ND	ND	ND	ND	ND
BTEX ^b	ND ^d	ND	ND	ND	ND	ND

^aPAHs = Polycyclic aromatic hydrocarbon. ^bBTEX = benzene, toluene, ethylbenzene, and xylenes. ^cND = none detected above the analytical detection limit of 2.7 mg/kg. ^dND = none detected above the analytical detection limit of 2 mg/kg.

The plots were planted with ornamental pineapples in February 2013, with a 1-m spacing between rows and a 0.3-m spacing between plants in each row. These areas were previously equipped with a drip irrigation system. The total amount of water applied during the year was 803 l/m² for the Groundwater and APO treatments, and 843 l/m² in the APF treatment. Irrigation was applied daily (approximately 2.3 l/m²), and water depths were calculated based on the estimated crop evapotranspiration and drainage losses measured with small-scale lysimeters (diameter = 0.4 m and depth = 0.7 m). All plots received the same amount of fertilizers, Ethrel flowering inductor (2-chloroethylphosphonic acid, 24% m/v), and other common practices in pineapple cultivation (Souza and Rheinhardt 2009).

The mesofauna was sampled 12 months after the start of irrigation with the three water treatments. For sampling, three undisturbed sub-samples of soil were collected in each plot using cylindrical PVC tubes (10 cm high and 10 cm diameter). After collection, the arthropods were extracted using a Berlese-Tullgren funnel. Arthropod mesofauna were counted and identified to order or suborder, and holometabolous insects were divided into adults and larvae. The oribatid mites were identified to the species level.

In controlled laboratory conditions at Embrapa Tropical Agroindustry (Fortaleza, Ceará, Brazil), we conducted two ecotoxicological tests using *Folsomia candida* Willem, 1902 (Collembola: Isotomidae) (ISO 11267, 1999) and *Enchytraeus crypticus* Westheide & Graefe, 1992 (Enchytraeida: Enchytraeidae) (ISO 16387, 2004) obtained from the Soil Fauna Laboratory of Embrapa Agrobiologia in Seropédica (RJ, Brazil). In the first assay, reproduction of these two species was evaluated in soil samples collected from the surface layer (0–0.1 m) of field plots (Groundwater, APO, and APF), after one year of irrigation in ornamental pineapple cultivation at FZB. In the second assay, we tested the reproduction of *F. candida* and *E. crypticus* on a non-irrigated soil sample collected from the pineapple field at FZB that received varying glutaraldehyde dosages in the laboratory (from 0.02 to 1000 mg/L). The concentration of glutaraldehyde in produced water treated by reverse osmosis used in this study was 0.198 mg/L, which represented the daily normal exposure of soil mesofauna. Thus, the exposures represented concentrations that were both less than and greater than the normal exposure of 0.198 mg/L.

For each of these two trials, we used four replicates, each with ten specimens kept in a plastic pot (diameter 4 cm and 7 cm high) containing 30 g of natural non-irrigated soils from the FZB (see page 85). The concentrations of glutaraldehyde (0.02, 0.05, 0.2, 0.4, 0.79, 10, 100, and 1000 mg/L) were prepared in deionized tap water and the soil was watered to 60% of its field capacity with each glutaraldehyde concentration. Approximately 2 mg of lyophilized yeast was provided as food for springtails and 4 mg of autoclaved oatmeal for the enchytraeids on the first and 14th days of the experiment. The temperature was $20 \pm 1^\circ\text{C}$, and the pH, electrical conductivity, and moisture were evaluated at the beginning and end of the tests according to the protocol validation criteria for the two species.

To evaluate whether an irrigation treatment affected individual mesofauna taxa, the analysis of indicator species (*IndVal*) proposed by Dufrêne and Legendre (1997) was used. *IndVal* values range from 0 to 1. Values closer to 1 indicate stronger associations of a species with a specific treatment. Significance values associated with *IndVal* were calculated by randomization tests. We did not include taxa with a frequency $\leq 10\%$ because that is a precondition for the *IndVal* analysis to be valid. Dominance ranking diagrams were constructed to evaluate changes in the patterns of dominance among the irrigation treatments. In the diagrams, the dominance gradient was represented by the X-axis and calculated from the relative abundance (on the Y-

axis) of each taxon collected in the irrigation treatments. We considered the groundwater to be the control treatment.

In ecotoxicological tests, the reproduction and survival of *F. candida* and *E. crypticus* were measured and the effect of glutaraldehyde evaluated using generalized linear models (GLM) modeled by the Poisson distribution. Effective concentration values (EC50) were calculated for organisms that had a significant decrease in their reproduction rates with glutaraldehyde by fitting the data to a logistic regression (log). In all analyses, we used the statistical program R (R Development Core Team 2014).

3 RESULTS AND DISCUSSION

We identified 18 taxonomic groups of arthropods in the three irrigation treatments evaluated (see page 84). Astigmata, Mesostigmata, and Oribatida mites were the most common organisms collected in the soil under both water-impacted treatments; however, Prostigmata mites occurred in small numbers. In this study, the greatest soil salinity disturbance occurred in the APF treatment. Astigmata are mites that rapidly colonize disturbed areas because of their rapid development and high reproductive rate (Philips 1990; Norton 1994). Predatory mites, such as Mesostigmata, have good dispersion rates and usually have a rapid lifecycle (Koehler 1999); therefore, they can colonize many impacted ecosystems (Lindquist et al 2009).

Five species of oribatid mites were found in all treatments. Among these species, only *Cosmochthonius* sp., commonly found in more arid environments, was significantly associated with one of the treatments, in this case, the Groundwater treatment (Table 2.2). Species of the family Cosmochthoniidae are relatively abundant in environments with more open vegetation in arid and semi-arid climates (Silva et al 1989; Penttinen and Gordeeva 2010), savannas (Santos et al 2008; Ferreira et al 2012), and agro-ecosystems (Osler and Murphy 2005). However, their abundance is higher in less contaminated areas (Osler and Murphy 2005).

Table 2.2. *IndVal* values for mesofauna of each group collected in the area cultivated with ornamental pineapple (*Ananas comosus* var. *erectifolius*) and submitted to three irrigation treatments.

Treatment	Taxa	<i>IndVal</i>	<i>p</i>	Frequency ^a (%)
Groundwater	Hymenoptera	0.53	0.01	29.63
Groundwater	Formicidae	0.17	0.62	18.52
Groundwater	<i>Cosmochthonius</i> sp.	0.44	0.02	14.81
Groundwater	<i>Lamellobates molecula</i> (Berlese, 1916)	0.20	0.53	11.11
APO	Coleoptera (larvae)	0.30	0.33	29.63
APO	Prostigmata	0.29	0.23	33.33
APO	Coleoptera (adult)	0.22	0.99	62.96
APO	Psocoptera	0.15	0.87	22.22
APO	<i>Galumna</i> sp.	0.28	0.15	14.81
APO	<i>Afronothrus</i> sp.	0.23	0.56	29.63
APO	<i>Archegozetes longisetosus</i> Aoki, 1965	0.10	1.00	11.11
APF	Entomobryomorpha	0.56	0.05	66.67
APF	Mesostigmata	0.45	0.18	81.48
APF	Astigmata	0.22	0.91	51.85

Numbers in bold represent significant *IndVal* values ($p \leq 0.05$). ^aCalculated as the percentage of samples where each taxon occurs.

A few organisms were related to one of the irrigation treatments, including the order Hymenoptera (excluding Formicidae), which occurred in the less impacted treatment (Groundwater). Some species of Hymenoptera that nest in the soil are sensitive to certain soil disturbances, such as physical impacts like trampling by animals (Bonte 2005), while others may be less abundant in soils with higher heavy metal concentrations (Nahmani and Rossi 2003). However, because some of the organisms were identified only to higher taxonomic levels which obscures any habitat partitioning that may be occurring at the species level (Franklin et al 2005), little can be explained about the effects of salinity in APF or the presence of glutaraldehyde in APO on these organisms. In the case of Collembola in the order Entomobryomorpha, which were affected by the APO, the glutaraldehyde used in this treatment may have affected their populations because they have greater sensitivity to various types of contaminants (Cortet et al 1999).

Although most taxa were not significantly different among the irrigation treatments, we observed changes in dominance patterns. In the Groundwater treatment, the four dominant groups were in the following order: Oribatida (*L. molecula*), Entomobryomorpha, Astigmata, and Mesostigmata. The species *Cosmochthonius* sp. and the order Hymenoptera (excluding Formicidae), which were

significantly associated with the Groundwater treatment (Table 2.2), were the sixth and the ninth most dominant groups, respectively (Figure. 2.1).

In the APO treatment, the pattern of dominance was the most different from the others. Astigmata, Oribatida (*A. longisetosus*), Mesostigmata, and Entomobryomorpha were dominant, although *A. longisetosus* was among the less dominant in the Groundwater treatment and did not occur in the APF treatment (Figure 2.1). Collembola of the order Entomobryomorpha were dominant in APF and were significantly associated with this treatment (Table 2.2). The Entomobryomorpha were the organisms that most changed in terms of dominance patterns, notably in APO.

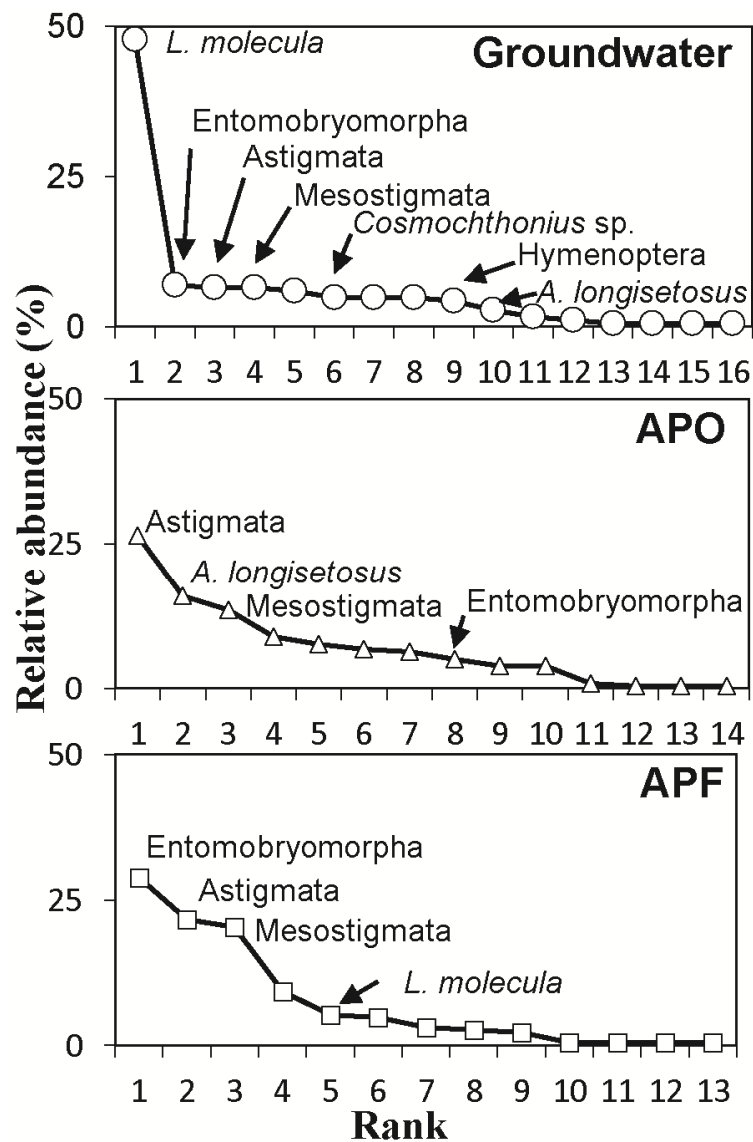


Figure 2.1. Dominance rank diagram of taxa collected in ornamental pineapple cultivation (*Ananas comosus* var. *erectifolius*) submitted to three irrigation treatments (groundwater, produced water filtered-APF and treated by reverse osmosis-APO). Only the most abundant organisms are highlighted.

Changes in dominance observed among the treatments can occur due to variations in biotic or abiotic conditions (Rae et al 2006; Gilbert et al 2009) or in the availability of resources. There were changes in habitat promoted by irrigation with APF (which is rich in salts) or APO (with the presence of biocides) in relation to the Groundwater treatment. This indicates that the irrigation treatments may have interfered with reproduction in some organisms, thereby changing the dominance patterns among taxa.

The ecotoxicological tests demonstrated that produced water had some effect on the reproduction of *F. candida* and *E. crypticus*, but not on their survival. In fact, the survival of an Enchytraeidae species and *F. candida* were not affected by high salinity (Owojori et al 2009). Despite the higher salt content in soil irrigated with APF than that in the other treatments, the reproduction of *F. candida* was significantly lower only in soil irrigated with APO ($p < 0.01$), probably because of glutaraldehyde. Reproduction in the APF treatment was similar to that in the Groundwater treatment ($p = 0.38$) (Figure 2.2).

In the case of *E. crypticus*, both APO ($p = 0.02$) and APF negatively affected the reproduction ($p = 0.02$) of these organisms (Figure 2.2). Terrestrial annelids like *E. crypticus* have more sensitive teguments and have direct contact with the soil solution (Laverack 1963). The long period of presence of glutaraldehyde in water may have concentrated that contaminant in the soil, and its effect, combined with other characteristics of APO, altered the physiology of these soil organisms. APF increased the salt content of the soil. Ions such as sodium and chlorine, can also adversely affect the reproduction of annelids, most of which are not tolerant to higher salt concentrations in the soil solution (Jänsch et al 2005).

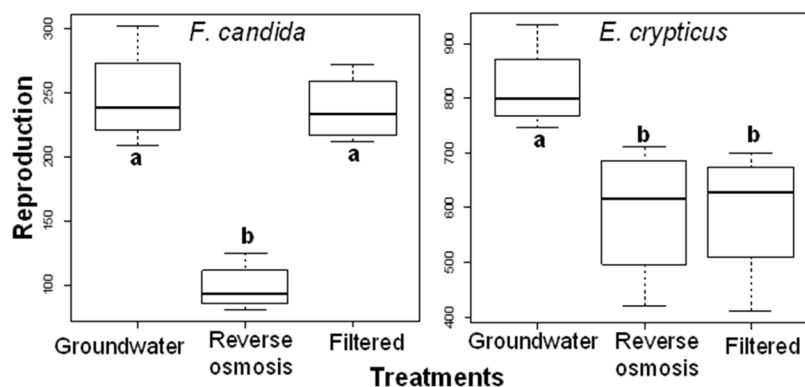


Figure 2.2. Reproduction of *F. candida* and *E. crypticus* on soil collected from three irrigation treatments. Identical letters indicate that treatments were not significantly different ($p > 0.05$).

Mortality in the control, juvenile production, and the coefficient of variation occurred within the range established by the protocols used. No significant adverse effects upon survival were observed in natural soil concentrations of up to 1000 mg/L of glutaraldehyde on *F. candida*. However, there was a negative impact on the reproduction during exposure to 100 ($p < 0.01$) and 1000 mg/L ($p < 0.01$) (Figure 2.3). The EC50 value for reproduction was 44.4 mg glutaraldehyde per liter of water in the soil.

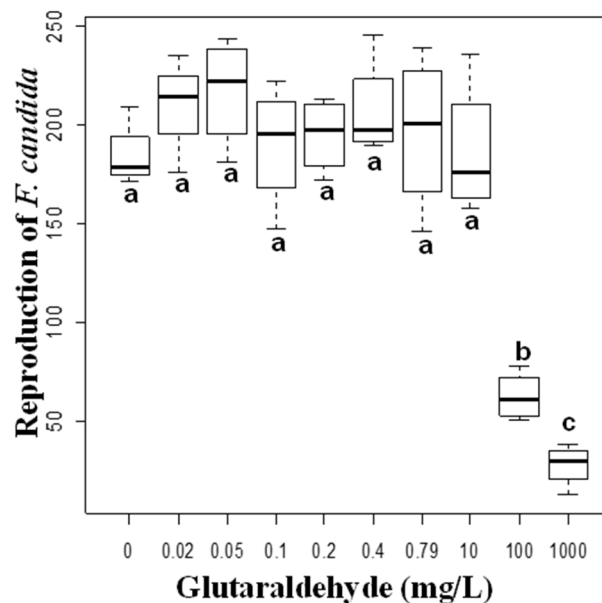


Figure 2.3. Reproduction of *F. candida* in natural soil watered at 60% of field capacity with different aqueous concentrations of glutaraldehyde. Identical letters indicate that treatments were not significantly different ($p > 0.05$).

Because it is a more common contaminant in wastewater (especially from hospitals) and in aquaculture, the ecotoxicological effects of glutaraldehyde have been evaluated for the survival of aquatic organisms (Leung 2001; Pereira et al 2014). In the present study, the EC50 value of glutaraldehyde at 44.4 mg/L for *F. candida* is higher than that found for effects upon reproduction in other arthropods. Reproduction tests showed a low concentration effect in crustaceans ranging from 4.25 mg/L for *Daphnia magna* Straus, 1820 (Leung 2001) to 4.90 mg/L (Sano et al 2005) for *Ceriodaphnia dubia* Richard, 1894. Thus, more glutaraldehyde in the soil was necessary to reduce the reproduction rate. There was no effect of concentrations up to 1000 mg/L of glutaraldehyde on the survival of *F. candida* in our study. The survival of invertebrates in glutaraldehyde was evaluated by other studies in aquatic organisms, and the only arthropods evaluated were crustaceans. In aquatic arthropod tests, the EC50 of

glutaraldehyde ranged from 0.11 mg/L for Copepoda to 582 mg/L for Amphipoda (see Pereira et al 2014).

The reproduction and survival of *E. crypticus* was not affected at a concentration of 1000 mg/L in the soil. Ecotoxicological tests with glutaraldehyde have been reported only for aquatic annelids, and these studies found that EC50 values were lower than 1000 mg/L. In survival tests with the aquatic oligochaete *Lumbriculus variegatus* Mueller, 1774, EC50 values were 11.1 (Sano et al 2003), and 6.3–16 mg/L (Sano et al 2004). In terrestrial environments, the organisms have less contact with water and glutaraldehyde is more easily degraded in soil (Leung 2001), thus a greater concentration may be required to affect the reproduction of the organisms evaluated.

Irrigation with produced water affected mesofauna identified at high taxonomic categories and oribatid mite species. The dominance relations among taxa were changed in soils irrigated with produced water from different treatment processes. Some mesofauna taxa exhibit a shift in the order of dominance when exposed to soils under irrigation with produced water, regardless of how the water was treated. Although the high doses of glutaraldehyde used in the treatment of reverse osmosis did not affect survival of the mesofauna, reproduction was clearly affected in both *F. candida* and *E. crypticus*. Further studies are needed to ascertain the causes of the observed mesofaunal dominance pattern shifts in soils irrigated with oil exploration wastewater.

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CAPÍTULO III

(Submetido ao periódico *Ecological Entomology*)

AVALIANDO O EFEITO DAS CONDIÇÕES AMBIENTAIS DO SOLO SOBRE ASSEMBLEIAS DE MICROARTRÓPODES: ESPÉCIES OU OUTRAS CATEGORIAS TAXONÔMICAS?

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Resumo - Investigações sobre os impactos da atividade agrícola no solo são realizadas utilizando a mesofauna como indicadora dessas perturbações. Para tanto, são abordadas desde identificações em nível de ordem, classe ou até espécies de alguns táxons de microartrópodes. O objetivo desse trabalho foi avaliar se a assembleia dos microartrópodes da mesofauna, identificados em classe ou ordem, responde de forma similar a assembleias de espécies, no caso ácaros Mesostigmata edáficos. Um ciclo de girassol e mamona no período seco e outro no chuvoso foram cultivados em uma área de semiárido. Utilizou-se água produzida proveniente da exploração petrolífera e água do subsolo captada do aquífero Açú na irrigação. Os microartrópodes foram coletados no solo sob as culturas, durante dois períodos sazonais, em três tratamentos de irrigação e em área de mata nativa. A abundância de microartrópodes e de espécies de ácaros correlacionaram-se, no entanto, a riqueza não apresentou correlação. As respostas da abundância e da riqueza das duas assembleias foram similares no girassol para ambas as condições avaliadas. Um grupo de microartrópodes (Diplura) associou-se ao tratamento de irrigação controle e nenhuma espécie de Mesostigmata pode indicar impacto da irrigação. Tanto os estudos com ordens de microartrópodes quanto de espécies de ácaros do solo possuem respostas semelhantes aos padrões de alteração do ambiente tais como a cobertura vegetal e a sazonalidade. No entanto, a estrutura da assembleia dos microartrópodes em geral é mais sensível aos impactos de irrigação do que a assembleia de ácaros Mesostigmata.

Palavras-chave: Monitoramento ambiental, Agroecossistemas, Biologia do solo, Semiárido.

ASSESSING THE EFFECT OF SOIL ENVIRONMENTAL CONDITIONS ON MICROARTHROPOD ASSEMBLAGES: DOES IDENTIFICATION TO SPECIES LEVEL OR TO A HIGHER TAXONOMIC RANK MATTER?

Abstract - Studies of the impact of agricultural activities on soil may be performed using microarthropods as indicators of soil disturbance. For that, taxonomic identification of microarthropods is usually done to the order, class, or even species level. The aim of this study was to evaluate whether the microarthropod assemblage identified to class or order levels responds to environmental variation in a similar manner to the assemblage identified to species level, in this case, edaphic Mesostigmata mites. Sunflower and castor bean cultures were planted in a semiarid area and followed during one dry and one rainy season. Produced water from oil exploration filtered and treated by reverse osmosis, and groundwater were used for irrigation. Microarthropods were collected in both crops and seasons, under three irrigation treatments, and in a native forest. The abundance of microarthropods and species of mites was correlated, although richness was not. The response of abundance and richness to environmental conditions was similar between assemblages in the sunflower crop. The microarthropod group Diplura was the only group affected by irrigation treatment. Both assemblages (all microarthropods and Mesostigmata mites) have similar responses to general environmental patterns, such as vegetation cover and seasonality. However, the general microarthropod assemblage seems to be more sensitive to irrigation impact than the Mesostigmata assemblage.

Keywords: Environmental Monitoring, Agroecosystems, Soil biology, Semiarid

1 INTRODUCTION

Owing to the expansion of agricultural frontiers and the impact caused by their management, environmental monitoring of soil quality has aroused the interest of research institutions and governments. Several methods have been proposed for the spatial and temporal monitoring of the microarthropod fauna, which consider that these organisms reflect soil conditions or disturbance (Ruf, 1998; Pik *et al.*, 2002). However, because of the high diversity of soil microarthropods and the techniques used for the taxonomic identification of these groups, identification of all organisms to the species level is time consuming and expensive (Giller, 1996; Santos *et al.*, 2008). These aspects are often responsible for not using microarthropods in environmental monitoring (Lawton *et al.*, 1998). In cases of high diversity, the selection of a particular taxonomic group such as mites (Santos *et al.*, 2008; Moraes *et al.*, 2011) may be an alternative, requiring less identification work. In addition, identification to a taxonomic rank higher than species has also been used because it is simpler and can be done more rapidly.

Identification of the microarthropod assemblage to the class, order or suborder levels is problematic because these taxonomic categories are very general and include organisms with different environmental responses, possibly masking the interpretation of species responses to environmental conditions (Franklin *et al.*, 2005). Furthermore, differences in taxonomic resolution between studies make comparison difficult since species composition is unknown, and therefore, beta diversity patterns are poorly understood (Prinzing *et al.*, 2003). However, this broad functional diversity approach may better reflect the ecosystem functions if a less diverse assemblage is used (Heemsbergen *et al.*, 2004).

Mesostigmatid mites are among the organisms that often compose microarthropod assemblages and that can be used in monitoring soil conditions (Ruf, 1998). Edaphic Mesostigmata are usually efficient predators in agroecosystems (Walter, 1988; Koehler, 1997, 1999) and occur together with other microarthropods on which they prey on (Walter & Proctor, 1998). This co-occurrence pattern suggests that mites and their prey respond to certain environmental conditions in a similar way.

Because there is no standard protocol for monitoring studies, environmental assessments are made using a variety of approaches, from using higher taxonomic categories to selecting a specific taxonomic group (Schmeller *et al.*, 2009; Schmeller

et al., 2015). As a result, interpretation of results and comparison among different studies is difficult (Gardner *et al.*, 2009). It is, therefore, important to understand how different taxonomic categories of soil microarthropod assemblages respond to certain agricultural practices such as irrigation.

The use of treated produced water from oil exploration for irrigation has many effects on the soil, like salinization, changes in its physical properties (Al-Haddabi & Ahmed, 2007), reduction of microbial activity (Lopes *et al.*, 2014) and reproduction of mesofauna (Ferreira *et al.*, 2015). The objective of the present study was to investigate the similarities in response to environmental variation between microarthropod assemblages identified at the order or class level and the fauna of edaphic mesostigmatic mites identified at the species level. We hypothesize that, most Mesostigmata being predators of other mesofauna taxa (Walter & Proctor, 2013), the interaction between these mites and other microarthropods may mean that both groups have similar responses to environmental change. Moreover, identification at higher taxonomic level, which is functionally more diverse, might be more sensitive to a range of environmental variations than identification at the species level, which is functionally less diverse.

2 MATERIALS AND METHODS

This study was conducted in a farm, Belém, located in the municipality of Aracati, State of Ceará, Brazil (4° 44' 4.23" S, 37° 32' 23.56" W). This region is characterized by a warm and semi-arid weather, with an annual rainfall of 800 mm. It has two distinct seasons: the rainy season between January and May and the dry season from June to December. The average temperature ranges from 26 to 28 °C. The soil of the experimental area was classified as Haplic Arenosol and the predominant vegetation is the seasonal tropical dry forest, known as Caatinga (Sampaio, 1995). Nearby the experimental plots is the oil exploration field of Belém farm, managed by Petrobras, that supplied water for the irrigation experiments.

A sample design of nine random subplots of 400 m² each were planted with oilseeds, namely castor bean (*Ricinus communis* L., BRS Energy) and sunflower (*Helianthus annuus* L., BRS 321). On a native area (approximately 100 m from cultivated area) of approximately 400 x 1000 m, nine sampling plots were established at approximately 130 m from each other. Both areas, with planted oilseeds and native

vegetation, were sampled during two successive cycles: first in the dry season (September 2012) and after in the rainy season (March 2013). Total rainfall was 1.8 mm in the dry season and 446.6 mm in the rainy one. Drip irrigation was used, whereby the water volume given per day was estimated by evaporation and drainage using lysimeter columns (0.4 in diameter and 0.7 in depth). During the two cultivation cycles, three different treatments were used, with three replicates each: a control treatment with groundwater from the Açu aquifer (200 m deep), a treatment with filtered produced water; and filtered produced water treated by reverse osmosis.

Soil microarthropods were collected in the two seasons (dry and wet), in each crop (castor bean and sunflower) and irrigation treatment (groundwater, filtered water, and reverse osmosis). A cylindrical soil sampling device (10 cm diameter × 10 cm depth) was used to collect six undisturbed soil subsamples in each plot (three during germination and three immediately before harvest), which were combined to form one sample by replicate. Subsamples were taken at a distance of seven meters from each other. In addition, two subsamples were collected in each of the nine plots established in the native vegetation area. Microarthropods were extracted from soil samples using the modified Berlese-Tullgren method (Franklin & Morais, 2006). Microarthropods that were part of the mesofauna were identified at the order level, whereby holometabolous organisms were divided between adults and larvae. Arachnids were divided into three groups: Araneae, Acari, and Pseudoscorpionida. Mesostigmata mites were identified at the species level.

Data were divided into two groups based on the taxonomic level used to identify the different assemblages. One included all microarthropods collected and identified to the taxonomic level of order or class, the other included only Mesostigmata mites identified to the species level. Richness, abundance, the relation of abundance and richness with environmental predictors (water irrigation and seasonality), and the effect of irrigation treatments on the individual taxa were compared between both assemblages. The structure of both assemblages in the cultivated and native vegetation areas was also assessed.

A Pearson correlation test with Bonferroni correction was used to assess whether the richness or abundance of Mesostigmata was correlated to richness or abundance of all microarthropods. The relationship of both groups of organisms with the seasonality and irrigation was analyzed using analysis of variance (ANOVA). For statistical analysis, data were transformed into $\log(x + 1)$. To assess whether some

taxa was associated with any of the irrigation treatments, the indicator species analysis (*IndVal*) proposed by Dufrene and Legendre (1997) was used (we did not include taxa with a frequency < 4). Differences in assemblage structure between irrigation treatments and crops seasonality, and between native vegetation and the cultivated area was analyzed using nonparametric multivariate analysis of variance (NP-MANOVA) using the Bray–Curtis index. A nonmetric multidimensional scaling analysis (NMDS) was used to generate a visual representation of the structure of both assemblages in native vegetation and cultivated area. The statistical program R (R Development Core Team, 2014) was used in all analyses.

3 RESULTS

In both cultivated and native vegetation plots, 15 species of Mesostigmata and 23 taxa of other microarthropods were identified during the dry and rainy seasons. In the native vegetation plots, 20 groups of microarthropods were present in both seasons, whereas eight species of mites were present only in the rainy season. In the cultivated area, 19 taxa of microarthropods and 10 species of mites were identified (Table 3.1).

Table 3.1. Mesostigmata mites and other microarthropods identified in three vegetation types.

Taxa	Relative abundance (%)			Relative frequency (%)		
	Sunflower	Castor bean	Native vegetation	Sunflower	Castor bean	Native vegetation
Acari	35.4	63.7	50.4	100	100	100
Araneae	3.28	1.28	0.25	77.8	100	44.4
Blattodea-Blattaria		0.04	0.38		11.1	33.3
Hemiptera-Sternorrhyncha	1.31	0.09		33.3	11.1	
Coleoptera (larvae)	10.6	3.76	0.44	100	100	55.6
Coleoptera	9.06	2.92	0.50	88.9	100	55.6
Dermaptera	0.13	0.09		11.1	22.2	
Diplura	1.05	0.35	0.19	44.4	55.6	33.3
Diptera (larvae)	3.94	1.24	1.26	77.8	77.8	66.7
Collembola	24.9	10.5	38.6	100	100	100
Hymenoptera-Formicidae	7.22	4.29	1.57	88.9	88.9	55.6
Hemiptera-Heteroptera	0.13	0.18	0.06	11.1	44.4	11.1
Hymenoptera (no formicidae)		0.04	0.06		11.1	1.11
Blattodea-Isoptera	0.39	1.28	0.06	22.2	33.3	11.1
Isopoda			0.06			11.1
Lepidoptera		0.04			11.1	
Lepidoptera (larvae)	0.13	0.09	0.06	11.1	11.1	11.1
Neuroptera (larvae)	0.13			11.1		
Orthoptera		0.04	0.76		11.1	44.4
Polyxenida			0.06			11.1
Pseudoscorpiones	0.26	0.04	1.70	11.1	11.1	88.9
Psocoptera	0.66	9.37	2.08	33.33	77.78	100
Symphyla			0.88			44.4
Thysanoptera	1.31	0.66	0.50	66.6	44.4	66.6
Zygentoma			0.13			22.2
Mesostigmata mites						
<i>Asca</i> sp.			43.3			0.54
<i>Cosmolaelaps</i> sp.			10.0			0.18
<i>Gaeolaelaps</i> sp. 1	50.0	25.8		88.9	100	
<i>Gaeolaelaps</i> sp.3			10.0			0.18
<i>Geolaelaps</i> sp. 2	2.50	2.44		22.2	33.3	
<i>Gamasellodes</i> sp.		0.98	23.3		22.2	0.36
<i>Gymnolaelaps</i> sp.			10.0			0.18
<i>Macrocheles</i> sp.	2.50	0.49		22.2	11.1	
<i>Neoseiulus</i> sp.		0.89			11.1	
<i>Oplitis</i> sp.	5.00	5.37		22.2	33.3	
<i>Proprioseiopsis</i> sp.			3.33			0.09
<i>Protogamasellopsis posnaniensis</i> Wisniewski & Hirschmann, 1991		20.5			22.2	
<i>Protogamasellus mica</i> Athias Henriot, 1961	40.0	32.7		88.9	77.8	
Uropodidae sp.3		10.3			11.1	
Uropodidae sp.4		0.49			11.1	

A correlation between both assemblages was found for abundance in the irrigated area (Pearson correlation = 0.91; $P < 0.001$), suggesting that they have a similar response to environmental predictors. However, the richness of the two groups was not correlated (Pearson correlation = 0.11; $P = 0.661$), indicating that the number of mite species does not reflect the amount of microarthropod taxa.

Differences in abundance and richness of Mesostigmata and all microarthropods between seasonal periods (dry and wet), crops (castor bean and sunflower), and irrigation treatments (aquifer, filtered and reverse osmosis) are shown in Table 3.2. The abundance and richness of mites and all microarthropod in the sunflower cultivation plots were not affected by any of the variables. However, in the castor bean plots, irrigation type and seasonal period affected the abundance of both assemblages. Unlike the mites, responses of all microarthropod were more complex due to the interaction between seasonal period and irrigation treatment. The richness of Mesostigmata was found to be influenced by seasonal period, while all microarthropods were influenced by irrigation. The responses of both taxonomic groups to the interaction between seasonal period and irrigation treatment were different.

Table 3.2. Analysis of variance on the effect of seasonal period and irrigation treatment on abundance and richness of Mesostigmata mites and other soil microarthropods.

Variables	Abundance				Richness			
	Mesostigmata mites		All microarthropods		Mesostigmata mites		All microarthropods	
	F	P	F	P	F	P	F	P
Sunflower N= 18								
Irrigation ^a	1.19	0.34	0.78	0.48	1.33	0.30	0.90	0.43
Season ^b	3.45	0.09	0.92	0.36	2.08	0.17	0.13	0.73
Season: Irrigation ^a	2.68	0.07	0.55	0.59	4.33	0.06	0.30	0.75
Castor bean N= 18								
Irrigation ^a	3.98	0.05	13.7	<0.01	2.02	0.18	11.1	<0.01
Season ^b	10.8	<0.01	9.12	0.01	7.20	0.02	3.03	0.11
Season: Irrigation ^a	2.62	0.11	6.02	0.01	2.47	0.13	0.03	0.97

^aF_{2,12}; ^bF_{1,12}. Significant values are represented in bold.

None of the Mesostigmata species was associated with the irrigation treatment. Among the all microarthropods identified to the order level, only Diplura

(*IndVal* = 0.63; *P* = 0.01) was related to groundwater, indicating the sensitivity of these organisms to water quality (Table 3.3).

Table 3.3. Indicator species analysis (*IndVal*) between irrigation treatment, and Mesostigmata species and all soil microarthropods. Individuals with lower frequency than four were not included.

Assemblages	Irrigation treatments	Taxa	<i>IndVal</i>	<i>P</i>
Mesostigmata mites	Groundwater	<i>Geolaelaps</i> sp.2	0.22	0.73
	Reverse osmosis	<i>Geolaelaps</i> sp.1	0.32	0.97
	Filtered	<i>Protogamasellus mica</i>	0.48	0.22
	Filtered	<i>Oplitis</i> sp.	0.42	0.19
All Microarthropods	Groundwater	Diplura	0.63	0.01
	Groundwater	Araneae	0.56	0.09
	Groundwater	Hemiptera - Heteroptera	0.53	0.06
	Groundwater	Hymenoptera - Formicidae	0.47	0.37
	Groundwater	Coleoptera	0.46	0.14
	Groundwater	Hemiptera - Sternorrhyncha	0.46	0.14
	Groundwater	Coleoptera (larva)	0.42	0.63
	Groundwater	Isoptera	0.24	0.57
	Reverse osmosis	Collembola	0.49	0.28
	Reverse osmosis	Thysanoptera	0.21	0.99
	Filtered	Diptera (larva)	0.62	0.06
	Filtered	Acari	0.57	0.20
	Filtered	Psocoptera	0.51	0.33

Significant values are in bold.

Overall, the responses of Mesostigmata assemblage were different in each type of crop. In the sunflower plots, both mites as well as all microarthropods were influenced by seasonal period. However, microarthropod assemblage was only influenced by irrigation when season was considered, indicating that the effect of irrigation depends on the season (Table 3.4).

Table 3.4. Nonparametric multivariate analysis of variance (NP-MANOVA) on the effect of seasonal period and irrigation treatment on the assemblage of soil Mesostigmata mites and all microarthropods using the Bray–Curtis index.

Variables	Sunflower				Castor bean			
	d.f.	Pseudo-F	R^2	P	d.f.	Pseudo-F	R^2	P
Mesostigmata mites								
Irrigation	2	0.35	0.03	0.76	2	2.46	0.29	0.02
Season	1	5.74	0.31	0.04	1	0.62	0.04	0.68
Season:irrigation	2	3.65	0.39	0.08	2	0.69	0.08	0.73
Residuals	5				10			
All microarthropods								
Irrigation	2	2.03	0.16	0.03	2	2.51	0.17	0.02
Season	1	5.57	0.21	<0.01	1	9.96	0.33	<0.01
Season:irrigation	2	2.18	0.17	0.02	2	1.68	0.11	0.09
Residuals	12				12			

Differences in structure of the two assemblages were found between the cultivated and native vegetation areas. In the native vegetation area, Mesostigmata did not occur during the dry season, and therefore, the two assemblages were only compared in the rainy season. Both the assemblage of Mesostigmata (Pseudo-F = 14.45; $R^2 = 0.42$; $P < 0.01$) as well as that of all microarthropods (Pseudo-F = 4.35; $R^2 = 0.18$; $P < 0.01$) were influenced by vegetation type. It was clear that the structure of both assemblages differed between the two types of land use, which indicated similarity in the responses of the two taxonomic approaches. Despite similar responses, the difference between analyzed areas was more evident when the assemblage of Mesostigmata was considered, suggesting a greater preference of Mesostigmata species for one of the areas (Fig. 1).

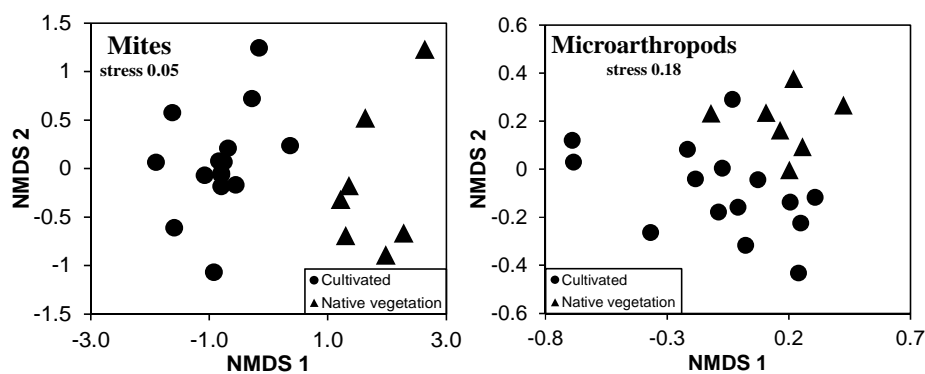


Figure 3.1. Nonmetric multidimensional scaling analysis (NMDS) of the structure of Mesostigmata mites and all soil microarthropods in two types of land use (cultivated and native vegetation) using the Bray-Curtis index.

4 DISCUSSION

The abundance of Mesostigmata was correlated with the abundance of all microarthropods. Nevertheless, there was little correlation in richness between the two groups. It is, however, known that most Mesostigmata are predators (except Uropodidae), and that they are more abundant in areas with higher abundance of potential prey such as springtails, other mites, or small immature insects (Walter & Proctor, 1998). For the same taxon, there is often high correlation between species richness and genera (Andersen, 1995; Prinzing *et al.*, 2003; Kallimanis *et al.*, 2012), families (Prinzing *et al.*, 2003; Kallimanis *et al.*, 2012), or even orders (Prinzing *et al.*, 2003) when all species of a group are compared with higher taxonomic categories. In the present study, this pattern differed probably because comparisons were made between different taxa (Schulze *et al.*, 2004), i.e., Mesostigmata mites were compared to arachnids and hexapods.

Overall, the abundance of the two microarthropod assemblages responded similarly to environmental variables in both the sunflower and castor bean cultivation plots (Table 2). Thus, a similar correlation in abundance between different assemblages indicated a similar response to environmental variation. In the castor bean plots, the abundance of all microarthropods was significantly related to interactions between variables, suggesting that the relationship between abundance of this group and environment conditions is complex. This complexity is probably due to the higher functional diversity of the all microarthropod assemblage (Dindal, 1990) than of Mesostigmata mites, which are predominantly predators (Walter, 1988; Koehler, 1999; Lindquist *et al.*, 2009). In fact, the responses of a community with higher functional diversity tend to be more complex, because it is not the number of species but the degree of functional differences between them that controls ecosystem processes (Heemsbergen *et al.*, 2004). In such cases, certain functional groups can, for example, be dependent on the season or type of vegetation, whereas others are not influenced by these variables (Rosenfeld, 2002). These types of responses result in statistically significant interactions, as found in the present study.

The richness of Mesostigmata and all microarthropods taxa responded similarly to environmental variation in sunflower cultivation plots, but differed in the castor bean plots. The response of the latter group is due to the low correlation in species richness of mites and taxa of all microarthropods, suggesting that low

correlation results in different environmental responses (Schulze *et al.*, 2004). The richness of Mesostigmata was more sensitive to seasonal period (instead of irrigation), than other taxa of microarthropods. When statistical analysis is done with data from groups identified to species level or to other low taxonomic rank, results show a more direct response to environmental variation than using groups identified to more general taxonomic categories (Bates *et al.*, 2007). Thus, using higher taxonomic ranks tends to mask the “real” richness, which does not occur if an assemblage is identified to the species level (Prinzing *et al.*, 2003; Franklin *et al.*, 2005). Therefore, understanding the response of richness to varying environmental conditions (e.g., seasonality) in higher taxonomic categories tends to be more difficult.

Diplura was the only group showing changes due to irrigation. In environmental monitoring studies, Diplura have been associated with areas with low human impact, such as soil compaction (Blasi *et al.*, 2013) or other types of agricultural management (Addison, 2007). However, to our knowledge, an effect of water quality on this group has not been reported yet. A relationship between Mesostigmata species and water quality was not observed in this study. When using taxonomic above species level, some information about the distribution of species that are within that same taxonomic category are obscured (Prinzing *et al.*, 2003). However, assemblages composed of various orders besides requiring less logistics for identification (Oliver & Beattie, 1993; Bates *et al.*, 2007), also have higher functional diversity than when using only Mesostigmata mites. In an assemblage with higher functional diversity, it is more likely that one of the taxa (Diplura in this study) shows changes in its distribution due to environmental variation (Andrén *et al.*, 1995). In the present study, the assemblage identified to the species level (Mesostigmata) did not allow an analysis of the effects of irrigation on richness or on the presence of an indicator species, probably because environmental variation was not enough to affect this functional group.

The Mesostigmata assemblage was less sensitive to irrigation in the sunflower cultivation plots and to seasonal period in the castor bean plots than all microarthropods. This indicates that in both crops, the all microarthropod assemblage reflected habitat conditions in terms of water quality better than the Mesostigmata assemblage (Table 4). Analyzing groups with higher functional diversity, such as the all microarthropod assemblage, is important because higher functional diversity can better reflect ecosystem functions (Heemsbergen *et al.*, 2004). Thus, the impact of

seasonality and irrigation management on the functionally more diverse assemblage may better reveal the impact of these practices on the function of the cultivated area.

Most variables influenced both the Mesostigmata and all microarthropod assemblages similarly (Figure 3.1). However, the response to irrigation in the sunflower crop, seasonality in the castor bean crop, and the interaction between variables for the microarthropod assemblage in the sunflower crop did not follow this pattern. Moreover, in the different types of land use (native vegetation × cultivated area), responses of Mesostigmata and all microarthropods were very similar but there was a clear difference in assemblages between areas indicating a higher specificity of the Mesostigmata to land usage type. In arid and semiarid environments, edaphic Mesostigmata are known to be sensitive to factors such as seasonality (Kinnear & Tongway, 2004), vegetation structure (Noble *et al.*, 1996), and agronomic practices of soil management (Bedano & Ruf, 2007). Other microarthropods are also influenced by the same factors in semiarid climates (Santos & Whitford, 1983). The sensitivity of the assemblages to these factors suggests that the two taxonomic groups approach can be used in this type of analysis and that species identification showed a higher habitat specificity of Mesostigmata.

We conclude that both studies, i.e., identification of microarthropod mesofauna assemblages to higher taxonomic ranks and of edaphic Mesostigmata mites to the species level, have similar responses to general environmental conditions, such as seasonality and vegetation cover. However, higher taxonomic categories, probably due to their greater functional diversity, have a stronger response to the various disturbances. Furthermore, identification of microarthropods to the level of order requires less time (Oliver & Beattie, 1993; Bates *et al.*, 2007) supporting the use of this approach in environmental monitoring. Overall, patterns of general environmental response between both assemblages are comparable.

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CONCLUSÕES FINAIS

A partir dos resultados deste estudo fica evidenciada a influência da irrigação com AP sobre a riqueza e a abundância de grupos taxonômicos de microartrópodes de solo. No entanto, tal influência depende do tipo de cobertura vegetal. A AP tratada por osmose reversa afeta a estrutura da assembleia dos organismos do solo independentemente do tipo de cultivo avaliado, porém, esse efeito é mais intenso no período de seca anual. A partir das perturbações provocadas pela AP, podem-se sugerir os microartrópodes no monitoramento ambiental da irrigação com tal água.

Além do efeito da AP, tratada por osmose reversa ou não, sobre a estrutura das assembleias de microartrópodes, esta pode ter efeito negativo sobre alguns táxons da mesofauna. Tais efeitos refletem uma alteração nos padrões de dominância dos grupos de organismos do solo. Não são observados apenas efeitos diretos da AP sobre os táxons, mas também do glutaraldeído utilizado no tratamento por osmose reversa, que afeta a reprodução de alguns organismos.

Não podem ser observados efeitos da AP em avaliações que utilizaram uma assembleia pouco diversa funcionalmente como os ácaros Mesostigmata. No entanto, quando se utiliza uma assembleia funcionalmente mais diversa, no caso de todos os microartrópodes de solo, identificados em nível taxonômico de ordem ou classe, o efeito da irrigação com AP se torna evidente. Sendo assim, os resultados de avaliações ambientais feitas com quaisquer grupos taxonômicos são comparáveis para padrões mais gerais do ambiente, como tipo de cobertura vegetal envolvida, mas não para irrigação.

CONSIDERAÇÕES FINAIS

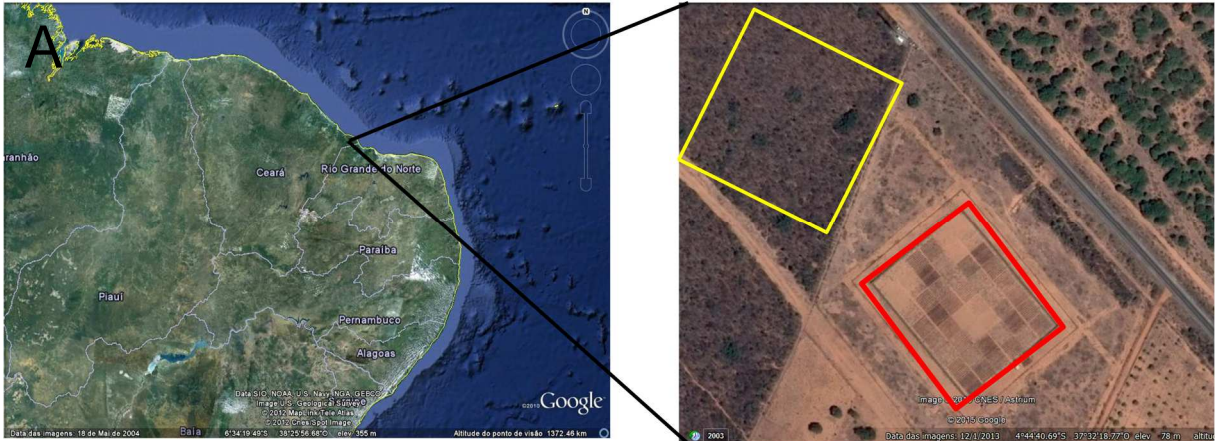
Atualmente, a sustentabilidade das atividades agrícolas tem se tornado tema de interesse mundial e a conservação dos solos é um aspecto importante para que a agricultura se mantenha produtiva durante longos períodos de tempo. Dentre os fatores que ajudam na manutenção da qualidade dos solos, está a biodiversidade dos organismos que habitam esse ambiente. Sendo assim, por avaliar diferentes aspectos da biota do solo sob impacto da irrigação por água produzida, esse trabalho procurou contribuir com o tema da sustentabilidade nos ambientes agrícolas do semiárido.

Observa-se, que os solos de regiões semiáridas, como o Nordeste brasileiro, são mais susceptíveis a perda de suas características físicas, químicas e biológicas, tornando-se salinos e abrindo caminho para a desertificação. A partir dessa fragilidade, os manejos das culturas agrônômicas como a irrigação devem ser monitoradas para que os impactos no solo sejam mínimos. Tendo em vista a importância dos organismos para a manutenção das funções dos solos, o entendimento dos impactos da irrigação sobre os microartrópodes pode fornecer subsídios para o manejo adequado dessa atividade.

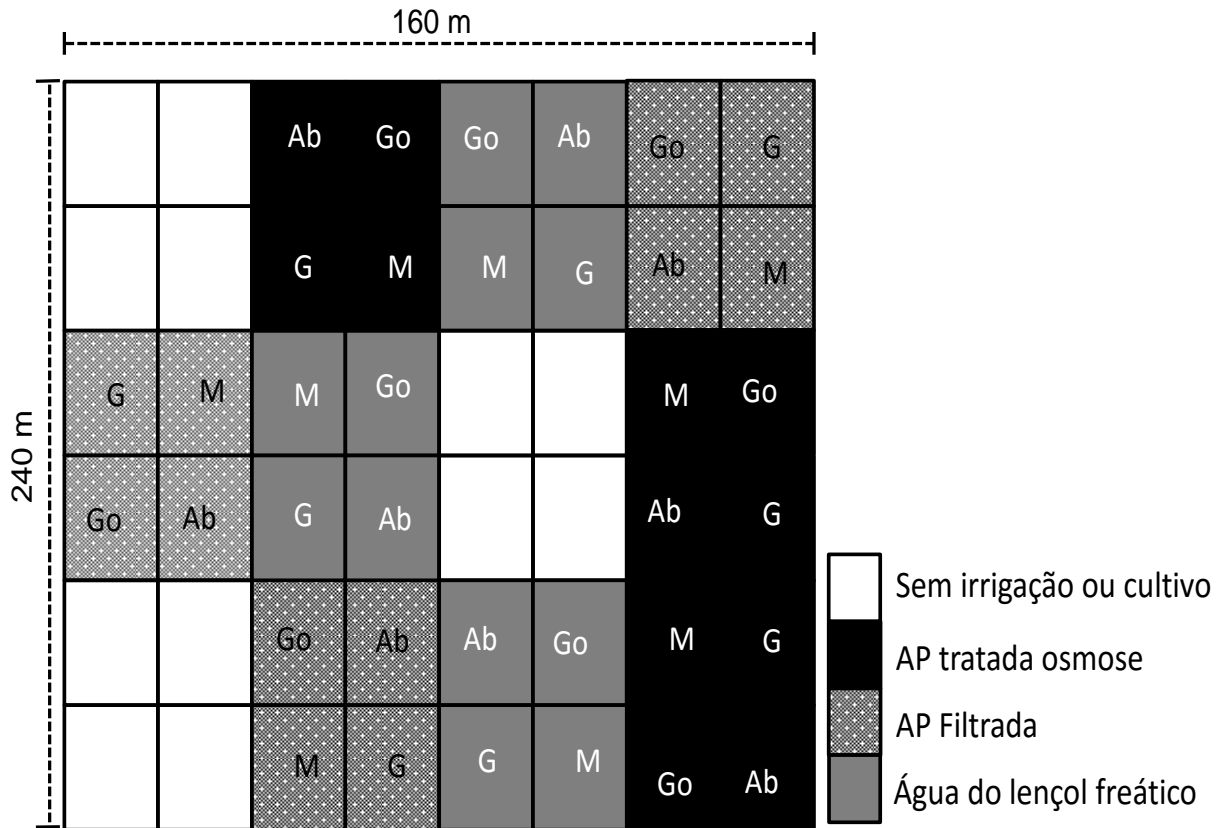
Os resultados obtidos nesse trabalho, juntamente com outros que ainda estão em andamento, ajudarão a compor banco de informações para uma avaliação criteriosa sobre o uso da AP na irrigação. Ressalta-se que as informações obtidas sobre os microartrópodes poderão ajudar em decisões como qual espécie vegetal cultivar ou se o cultivo deve ocorrer em apenas um dos períodos sazonais ou nos dois. Dados de outros trabalhos envolvendo a composição química do solo, a microbiota e como a decomposição do material vegetal é afetada pela AP poderão ser correlacionados com a abundância, riqueza e estrutura da assembleia de microartrópodes. Essas correlações fornecerão uma noção de como utilizar melhor os microartrópodes nas avaliações de impacto da AP no ambiente.

ANEXOS

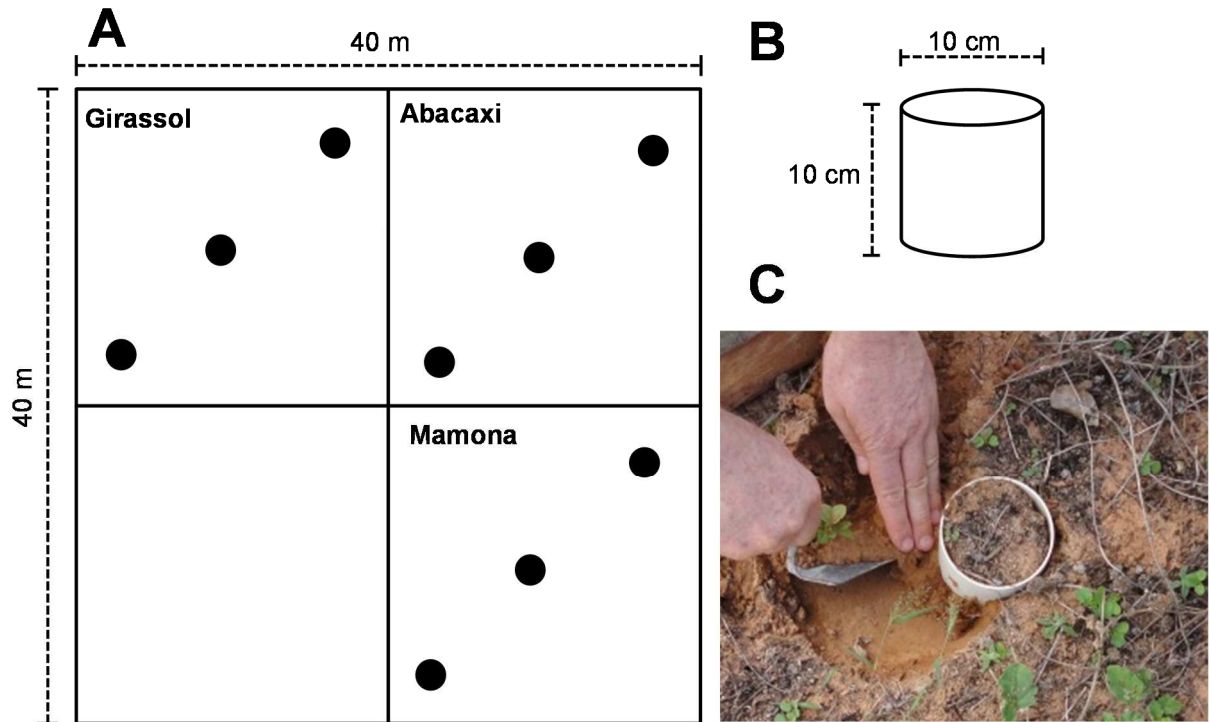
Anexo 1. Imagens de satélite obtidas através do programa Google Earth® evidenciando na área de estudo a vegetação nativa (quadrado amarelo) e a área de cultivo (quadrado vermelho) (A). Aspecto da vegetação antes da implantação do experimento de irrigação (B).



Anexo 2. Delineamento experimental implantado na Fazenda Belém em Aracati-CE para avaliar o efeito da irrigação com água produzida sobre as culturas de Mamona (M), Girassol oleífero (G), Girassol ornamental (Go) e Abacaxi ornamental (Ab).



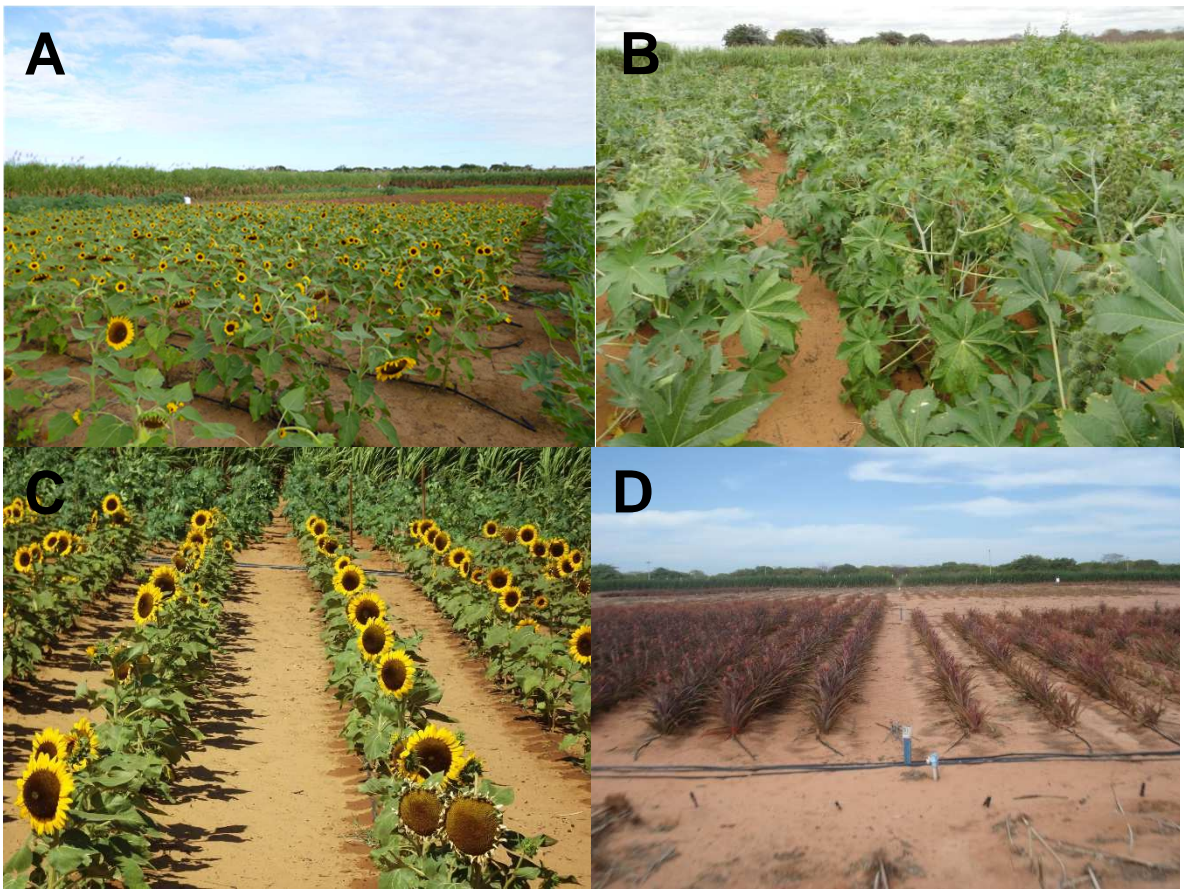
Anexo 3. Esquema de amostragem de solo para extração dos microartrópodes (os pontos pretos de evidenciam os locais de amostragem) (A), as dimensões da sonda utilizada na amostragem (B) e processo de amostragem (C).



Anexo 5. Imagem da área experimental antes (A) e durante o cultivo das espécies vegetais avaliadas (B).



Anexo 6. Cultivo de girassol ornamental (A), mamona (B) girassol oleífero (C) e abacaxi ornamental (D).



Anexo 7. Abundância de espécies de ácaros oribatídeos coletados na área cultivada com mamona, girassol e na vegetação nativa.

Espécies	Estação seca			Estação chuvosa		
	Mamona	Girassol	Vegetação nativa	Mamona	Girassol	Vegetação nativa
<i>Aphelacarus acarinus</i> (Berlese, 1910)			9			14
<i>Atropacarus</i> (<i>Hoplophorella</i>) <i>hamatus</i> (Ewing, 1909)			4			72
<i>Cosmochthonius</i> <i>lanatus</i> (Michael, 1885)			2			
<i>Epilomania</i> sp.			4			9
<i>Eremaeozetes</i> sp.						28
<i>Galumna</i> sp.						39
<i>Globoppia</i> sp.1			11			13
<i>Haplochthonius</i> sp.			3			9
<i>Liodes</i> sp.			4			10
<i>Peloribates</i> sp.			3			18
<i>Pergalumna</i> sp.1						169
<i>Pergalumna</i> sp.2			2			13
<i>Pergalumna</i> sp.3						14
<i>Rhysotritia</i> sp.1			6			
<i>Scheloribates</i> sp.			51			
<i>Teleoliodes zikani</i> (Sellnick, 1930)						10

Anexo 8. Abundância relativa dos microartrópodes coletados em cada tratamento de irrigação no cultivo de abacaxi ornamental.

Taxa	Aquífero	Osmose	Produzida
Ácaros oribatídeos (Oribatida)			
<i>Lamellobates molécula</i> (Berlese, 1916)	47,85		4,87
<i>Afronothrus</i> sp.	0,54	7,56	3,10
<i>Archezogozetes longisetosus</i> Aoki, 1965	2,69	15,97	
<i>Galumna</i> sp.		6,30	9,29
<i>Cosmochthonius</i> sp.	4,84		
Demais microartrópodes			
Entomobriomorpha	6,99	5,04	28,76
Astigmata	6,45	26,47	21,68
Mesostigmata	6,45	13,45	20,35
Coleoptera	5,91	3,78	5,31
Hymenoptera-Formicidae	4,84	0,84	0,44
Psocoptera	4,84	8,82	0,44
Hymenoptera (sem Formicidae)	4,30	0,42	0,44
Coleoptera (larva)	1,61	6,72	2,21
Prostigmata	1,08	3,78	2,65
Araneae	0,54	0,42	
Diptera	0,54		0,44
Symphipleona	0,54		
Orthoptera		0,42	

Anexo 9. Testes ecotoxicológicos avaliando a reprodução de *E. crypticus* e *F. candida* em solo irrigado com água produzida e solo contaminado com glutaraldeído.

