Ecological Indicators 48 (2015) 55–62

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Testate amoebae colonizing a newly exposed land surface are of airborne origin

Manfred Wanner[a,*], Michael Elmer[b], Michael Sommer[c,d], Roger Funk[c], Daniel Puppe[a,c]

[a] Brandenburg University of Technology Cottbus-Senftenberg, Dept. General Ecology, 03013 Cottbus, Germany
[b] Brandenburg University of Technology Cottbus-Senftenberg, Forschungszentrum Landschaftsentwicklung und Bergbaulandschaften (FZLB), 03013 Cottbus, Germany
[c] Leibniz-Centre for Agricultural Landscape Research (ZALF) e.V., Institute of Soil Landscape Research, 15374 Müncheberg, Germany
[d] University of Potsdam, Institute of Earth and Environmental Sciences, 14476 Potsdam, Germany

ARTICLE INFO

Article history:
Received 6 March 2014
Received in revised form 24 July 2014
Accepted 27 July 2014

Keywords:
Artificial water catchment
Immigration by air
Biological indicator

ABSTRACT

We hypothesized that at the very beginning of terrestrial ecosystem development, airborne testate amoebae play a pivotal role in facilitating organismic colonization and related soil processes. We, therefore, analyzed size and quantity of airborne testate amoebae and immigration and colonization success of airborne testate amoebae on a new land surface (experimental site "Chicken Creek", artificial post-mining water catchment). Within an altogether 91-day exposure of 70 adhesive traps, 12 species of testate amoebae were identified to be of airborne origin. *Phryganella acropodia* (51% of all individuals found, diameter about 35–45 μm) and *Centropyxis sphagnicola* (23% of all individuals found, longest axis about 55–68 μm), occurred most frequently in the adhesive traps. We extrapolated an aerial amoeba deposition of 61 individuals d^{-1} m^{-2} (living and dead individuals combined). Although it would be necessary to have a longer sequence (some additional years), our analysis of the "target substrate" of aerial immigration (catchment site) may point to a shift from a stochastic (variable) beginning of community assembly to a more deterministic (stable) course. This shift was assigned to an age of seven years of initial soil development. Although experienced specialists are necessary to conduct these time-consuming studies, the presented data suggest that terrestrial amoebae are suitable indicators for initial ecosystem development and utilization.

1. Introduction

Organismic immigration, colonization and resulting succession (or community assembly) are key events in initial ecosystems. Unicellular protists (including testate amoebae) are among the first heterotrophic eukaryotes arriving at newly exposed land surfaces, facilitating the establishment of plants and animals by improving the availability of nutrients. These early colonizers of "sterile" substrates are predominantly arriving by air (discussed in Hodkinson et al., 2002). General investigations about airborne pathogenic and free living amoebae (Rivera et al., 1994) and other eukaryotic microorganisms (Schlichting, 1969) revealed the importance, but also limitations of wind dispersal for passive transportation (e.g., Foissner, 2008; Lara et al., 2011; Rogerson and Detwiler, 1999; Smith et al., 2008; Smith and Wilkinson, 2007; Wilkinson, 2001; Wilkinson et al., 2012; Yang et al., 2010). Important airborne colonizers of new terrestrial substrates are testate amoebae. They form a polyphyletic group of abundant unicellular heterotrophic protists producing a shell that evolved independently. Their shells can be proteinaceous or agglutinated. Amoebae with agglutinated shells comprise xenosomic and idiosomic taxa building up their shells from foreign materials (so-called xenosomes, e.g., mineral particles) or endogenous self-synthesized calcareous or siliceous platelets (so-called idiosomes), respectively (details in Meisterfeld, 2002a,b). Testate amoebae establish in the new substrate within a short time span (Wanner et al., 1998; Wanner et al., 2008; Wanner and Xylander, 2005). The aforementioned authors analyzed testate amoebae after their arrival at the experimental plots, where amoebae already reproduced. Consequently, there are no data available on actual quantities of testate amoebae in the air. In the light of the debate on biogeographical distribution of testate amoebae (e.g., Finlay, 2002; Foissner, 2006), especially this information is of interest.

Wilkinson et al. (2012) used computer simulations to analyze the probability of global aerial dispersion of microorganisms with diameters from 9 to 60 μm. They found a distinct

* Corresponding author. Tel.: +49 355 69 2738.
E-mail addresses: wanner@b-tu.de, wanner@tu-cottbus.de (M. Wanner).

http://dx.doi.org/10.1016/j.ecolind.2014.07.037
1470-160X/

relationship between size and theoretical (global) dispersion. However, no practical studies on testate amoebae and their size-dependent transportation by wind were conducted. The wind dispersal of testate amoebae is subject to the same aerodynamic principles as any other aerosol suspended in the air, as mineral dust, soot or pollen. Regarding the size and density, testate amoebae are part of the 'short term suspension' transportation mode, affecting particles with diameters between 20 and 70 μm. They can remain suspended in the air for several hours and be transported some hundreds of kilometers (Shao, 2000). Consequently, the possible source areas of testate amoebae detected at a target can cover large regions.

The artificial water catchment "Chicken Creek" is a suitable investigation site, since interdisciplinary investigations started from "point zero" of construction (Gerwin et al., 2009, 2010, 2011; Wanner and Elmer, 2009). Up to now, limited knowledge exists about the very initial phase of ecosystem development, although this stage may play a crucial role for the later stages of system development (Hüttl et al., 2014; Raab et al., 2012). Esperschütz et al. (2013) proved the importance of fungal abundance and activity for litter decomposition in the initial phase. Further, a rapid immigration of biota in the Chicken Creek catchment was documented, followed by a distinct increase in trophic links within the soil food web in the first years of ecosystem development. Above-ground (plants) and below-ground succession (soil biota) were strongly correlated (Elmer et al., 2013). A study on inland dunes revealed also a pronounced relationship between plant successional stage and amoebal community assembly (Wanner and Xylander, 2005), pointing to a strong bio-indicative potential of terrestrial testate amoebae.

We, therefore, hypothesized that at the beginning of terrestrial ecosystem development, airborne testate amoebae play a pivotal role in facilitating organismic colonization and related soil processes. Thereby, they could act as indicators for initial ecosystem development and utilization. However, numbers and species of airborne soil organism immigration as well as their colonization success remain uncertain. Thus, the aims of our work were to analyze

(i) deposition of airborne testate amoebae ("amoeba rain") and
(ii) immigration and colonization success of airborne testate amoebae on a new land surface.

2. Material and methods

2.1. Study site

The study site Chicken Creek (51°36′18″N, 14°15′58″E) (Fig. 1) is part of a post-mining landscape located in the active mining area "Welzow-Süd" (lignite open-cast mining, 150 km south-east of Berlin) in the state of Brandenburg, Germany (Kendzia et al., 2008). Climate is characterized by an average air temperature of 9.3 °C with an annual precipitation of 559 mm comprising data from 1971 to 2000 (Gerwin et al., 2011). The construction as an artificial catchment of 6 ha with an altitudinal difference of about 15 m between the north-western (140 m a.s.l.) and south-eastern part (125 m a.s.l.) was completed in September 2005 ("time zero"). The initial substrate for soil development comprises a 2–3 m thick sandy, lignite- and pyrite-free Quaternary sediment. The sediments were taken from a depth of 20–30 m during lignite mining process and are characterized by sands to loamy sands with low carbonate contents (Gerwin et al., 2009). A weather station, located within the study site, continuously measured wind direction and speed two meters above ground (Biemelt and Nenov, 2010). Due to the elevated exposition and open character of the Chicken Creek study site, relatively steady (99% durance) and strong winds occurred (speed above 4.4 m s^{-1} during 24% of measuring period). Main wind direction is from the west-southwest (Biemelt and Nenov, 2010) (Fig. 1). Numerous studies have been conducted with respect to ecosystem development (e.g., Elmer et al., 2013; Gerwin et al., 2011; Zaplata et al., 2011) on this artificial catchment. The study on hand is based on a survey from Wanner and Elmer (2009).

2.2. Analysis of airborne testate amoebae

"Seed rain" traps were installed on the catchment edge with a distance of 20 m between each sampling point (Zaplata et al., 2011) (Fig. 1). A seed rain trap is a disposable petri dish (150 mm in diameter) provided with filter paper attached to the petri dish with paraffin-based bag balm and also covered with it. These petri dishes contained no growth media and were meant as passive, sticky traps for airborne organisms. The sticky surfaces of the traps were almost horizontally oriented (alternated in pointing slightly in- and outwards of the catchment) at about a height of 0.3 m (Zaplata et al., 2011). These traps were originally used by Zaplata et al. (2010, 2011) to trap wind-transported plant seeds. Since all kinds of small particles, also testate amoebae, adhered to the sticky surfaces, we used these traps for our investigations, too. Adhesive traps installed at the western border of the catchment (exposed to the main wind direction) were considered as input zone ($n = 19$, IN); traps installed at the eastern border of the catchment were considered as output zone ($n = 21$, OUT) (Fig. 1). With respect to calculations and figure preparation, main data basis is the immersion oil approach. The other methodological approaches (water instead of immersion oil, supplementary exposition time) served for method testing and search for further amoebae species (details in Table 1).

An Olympus BX50 bright field transillumination microscope was deployed to scan three cover slip areas (18 mm × 18 mm each) per adhesive trap for testate amoebae. Since adhered testate amoebae were dried out, the shells were filled with air and formerly living or encysted amoebae ("full" shells) could not be separated from dead ones ("empty" shells). Immersion oil (Olympus, Japan) proved to be the best embedding medium, resulting in transparent, artifact-free samples (cf. Section 3.1). In some cases, only the amoeba genus could be determined, because it was not possible to turn the amoeba shell necessary for species determination. Microscopical investigation (200×) was difficult, because the fibrous structure of the filter paper required about one hour of microscopy per cover slip. A total of 330 cover slips had been analyzed, of which 120 were used for a more detailed analysis (for details on the sampling scheme, see Table 1). With respect to the third study period from July to September 2009 (42-day exposition) (Table 1), in a first approach water was used to connect the sticky trap surface with the glass slide (cover slip), and in a second (more successful) approach, immersion oil. Here, different locations within the adhesive trap surface were scanned microscopically (for details on the sampling scheme, see Table 1).

Generally, detection of a single individual on a sticky trap was recorded, that means, there is no threshold number of individuals needed for analysis.

2.3. Soil sample analyses (target substrate)

Soil sampling and subsequent laboratory procedures followed Wanner and Elmer (2009): Substrate samples from Chicken Creek catchment were removed with a spatula (five samples per site pooled, each sample 50 mm^2 × 3 mm depth) from 24.08.2009 to 07.09.2012 (Table 1, Table 2) from vegetation covered spots (mainly *Trifolium arvense*). In the laboratory, 2 g of fresh substrate were transferred to 8 ml formalin (4% aqueous formaldehyde solution) for conservation. Aniline blue was added to differentiate between