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Toward the use of testate amoeba functional traits as indicator of floodplain restoration success

Bertrand Fournier [a,b,c,*], Elena Malysheva [d], Yuri Mazei [d], Marco Moretti [e], Edward A.D. Mitchell [a,b,c]

[a] *Laboratory of Soil Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2000 Neuchâtel, Switzerland*
[b] *Swiss Federal Research Institute WSL, Community Ecology, Wetlands Research Group, Station 2, CH-1015 Lausanne, Switzerland*
[c] *Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratory of Ecological Systems, Station 2, CH-1015 Lausanne, Switzerland*
[d] *Department of Zoology and Ecology, Penza VG Belinsky State Pedagogical University, Penza 440026, Russia*
[e] *Swiss Federal Research Institute WSL, Community Ecology, Insubric Ecosystems Research Group, CH-1015 Lausanne, Switzerland*

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A B S T R A C T

Functional traits (FT) offer a new framework to understand the ecology of organisms and overcome taxonomic difficulties that currently limit the study of minute soil taxa. FT are likely to be selected by environmental filters and hence they may provide more direct information on ecosystem characteristics than the species composition of a community.

We tested the potential of testate amoeba (TA) functional traits as bioindicators of selected ecosystem processes in the context of a restored floodplain in north-western Switzerland. The floodplain was divided into six functional process zones (FPZs) associated to distinct post-restoration successional stages. We selected TA FT and computed three functional indices: functional richness (FRic), divergence (FDiv), evenness (FEve), and dispersion (FDis). We then compared the patterns of functional indices and classical diversity indices such as species richness, diversity and evenness. We assessed whether traits converged or were over-dispersed in the different FPZs using a randomization procedure. Finally, we related environmental variables and functional traits using the "Fourth Corner" statistic. This procedure enabled us to highlight relations that can potentially be used for bioindication. Promising candidates include the relationships between shell biovolume and vegetation structure and between shell compression and plant litter input variables.

1. Introduction

A basic assumption of functional ecology is that differences in traits of species imply differences in the functioning of the ecosystem. Relating functional traits (FT) to environmental variables may improve our understanding of biological processes in ecosystems and allow defining a general and useful theory of species assembly [1]. The rationale for this approach is that FT are likely to be selected by environmental conditions and hence analysing them provides more direct information on ecosystem characteristics than the species composition of a community. Functional traits and measures of community functional diversity provide a way to overcome taxonomic limitations that are especially critical for minute soil taxa and tend to correlate more strongly than traditional species diversity with ecosystem functions such as productivity [2], resilience to perturbations [3], or regulation of biogeochemical fluxes [4].

Soil micro-organisms may differ from above-ground communities with respect to their resistance, resilience, dispersal potential, and adaptation strategy [5,6]. Characterizing the distribution of FT along environmental gradients may help to understand the causes of the different response to perturbation of above- and below-ground organisms. Our focus here is on testate amoebae (TA) FT in the context of floodplain restoration.

After centuries of increasing human impact on rivers (e.g. embankment, flood regulation, etc.), many floodplains are being actively restored. However, restoration projects often do not include monitoring of restoration success and there is currently no consensus on which indicators should be used to assess restoration success [7,8]. Species data are often used in biomonitoring but because restoration projects are being carried out in different regions, differing in their floras and faunas, specific protocols need to be defined for each biogeographical region. A biomonitoring

* Corresponding author. Laboratory of Soil Biology, University of Neuchâtel, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland.
E-mail addresses: bertrand.fournier@unine.ch (B. Fournier), elenamalysheva@list.ru (E. Malysheva), yurimazei@mail.ru (Y. Mazei), marco.moretti@wsl.ch (M. Moretti), edward.mitchell@unine.ch (E.A.D. Mitchell).

approach based on functional traits may thus allow overcoming biogeographical limitations.

TA are a polyphyletic group of free-living protozoa that play important roles in soil nutrient cycling [9] and especially the cycles of C, N, and Si in soils [10,11]. As for many minute to microscopic soil organisms [12] the taxonomy of is poorly resolved [13] and this may undermine their use as bioindicators if species-level identification is required [14]. The solution usually applied in ecological and palaeoecological studies is to lump species in morpho-taxa and species complexes [15].

Here we explore another possible way to overcome this limitation by using species functional traits. TA produce shells that differ in their composition, size and shape [16]. These morphological differences are believed to represent adaptations to the soil environment especially the soil moisture regime. As the soil moisture regime is controlled by several factors including soil particle size distribution, organic matter content, vegetation strata structure, litter input, elevation (and hence water table depth and frequency of flood), these different variables may influence TA species traits distribution.

TA are divided into two phylogenetically distinct groups, the Euglyphida (Rhizaria) [17] and the Arcellinida (Amoebozoa) [18]. Morphological adaptation to the soil (i.e. shell compression and aperture in a ventral position) appeared independently in both groups [19,20]. Thus TA represent an interesting example of evolutionary convergence and hence an ideal test group for linking phylogeny and functional ecology.

The functional importance of TA species traits in ecosystems is poorly understood. Attempts to link TA species traits to environmental gradients are limited to using the ratio of Arcellinida to Euglyphida (or Lobose/Filose amoebae index) [21,22]. Arcellinida (Lobose TA) are assumed to be *K*-strategists while Euglyphida (Filose TA) are considered as *r*-strategists. Higher *L*/*F* ratios are usually recorded in more stable and/or more developed ecosystems or microhabitats.

We explore the potential of TA FT as indicator of ecosystem functions in a recently restored floodplain. First, we selected traits and tested whether they were convergent due to environmental filters or divergent due to competition. We then classified a set of environmental variables according to the ecosystem function they are related to. We then used the existing methods to relate functional traits to environmental variables directly. We hypothesized that the composition and functions of TA communities differ among habitats, and that trait convergence is stronger in the more dynamic zones. We also expected that traits related to the origin or shape of shell material will be the most useful indicators of ecosystem process since they likely reflect adaptations of TA to environmental settings.

2. Material and methods

2.1. Study area

The study site is a 1.5 km long stretch along River Thur near Frauenfeld, Switzerland (365 m asl; annual precipitation: 1000 mm year^{-1}; average annual temperature: 7.9 °C). The site was restored in 2002 through riverbed widening from 50 to 150 m. The major banks were stabilized by plantations of willows (*Salix viminalis*). Further information on this site is given in Woolsey et al. [23]. We selected six habitats based on the functional process zones (FPZ) [24] proposed by Samaritani et al. [25] to represent the different post-restoration successional stages present at the site. These zones represent a gradient of decreasing flood mechanical impact. Closest to the river, FPZ-1 ("Tall herbs") was characterized by dense vegetation dominated by *Phalaris arundinacea*. FPZ-2 "Willow bush" was dominated by *S. viminalis* bushes (planted at the time of the restoration). The three forested FPZs were discriminated based on the dominant tree species: FPZ-3 (Forest) & FPZ-4 (Mixed forest): *Acer pseudoplatanus* and *Fraxinus excelsior*, with higher overall cover in FPZ-4. FPZ-5 (Willow forest): *Salix alba*. The reference FPZ-6 ("Pasture") is located directly upstream from the restored area and represents the pre-restoration "control". All soils were described as FLUVIOSOLS [26] with soil depth increasing from the most dynamic to the most stable forest FPZs.

2.2. Sampling methods

Study plots were selected in representative areas of the FPZs: six replicates were used in the more dynamic FPZs (Tall herbs, Willow bush, and Forest), and four in the more stable ones (Mixed forest, Willow forest, and Pasture) representing a total of 30 plots. Each plot consisted in an 8-m diameter circle. Coordinates and elevation of the centre of each plot were measured with a Differential GPS.

Environmental variables related to different ecosystem functions (Table 1) were measured in each plot. For Organic (OC) and total carbon (C), and total nitrogen (N) measurements, three cores of 10 cm depth and 6 cm diameter were extracted at each sampling site, homogenized and sieved at 2 mm. Basal respiration (BR) was estimated using Infrared Gas Analyser (Licor 8100). Fresh soil samples were left at room temperature for at least 3 h and then the CO_2 emissions from 40 g of fresh soil placed in Licor 8100-102 survey chambers were monitored for 9 min. All CO_2 emissions measured were highly stable ($R^2 > 98\%$).

For TA sampling, all litter and soils of the uppermost 5 cm were sampled in a $\sim$10 m^2 transect perpendicular to the river within each plot. In order to remove large debris, this material was sieved in the field (mesh = 1 cm). TA were extracted from subsamples of the remaining homogenized material by sieving through 0.5 mm mesh (see [27] for details) and then counted and identified [28,29] under light microscopy.

2.3. Functional traits

Five functional traits were selected according to their potential significance for ecosystem functions (Table 2):

1) Phylogenetic grouping (binary: Euglyphida = 0, Arcellinida = 1) may imply different functions in the ecosystem and different evolutionary stable strategy to cope with environmental settings [30,31].
2) The origin of the material used for test construction (binary: Agglutinate = 0, Secreted = 1) may allow an environmental filter to operate, in relation to the availability of the different substrates (e.g. mineral particles of adequate size, fungal hyphae, silica) or the relative cost of building a self-secreted shell by comparison with an agglutinated one.
3) The position of the aperture reveals a gradient from completely exposed to completely cryptic (semi-continuous coded as continuous: 1 = Axial aperture, 2 = Acrostomic, 3 = Plagiostomic, 4 = Cryptostomic aperture). Increasing protection of the aperture is generally interpreted as an adaptation to decreasing soil moisture content.
4) Test compression (binary: 0 = non-compressed, 1 = compressed) is also interpreted as an adaptation to living in a thin water film and allowing the amoebae to remain active longer when the soil moisture content decreases.
5) Biovolume (continuous: μm^3) may be constrained or enhanced by given environmental conditions (soil moisture, pore size). It was calculated based on size measurement data (length or

Table 1
List of the environmental variables measured at each plot.

Category	Type of data	Code	Variable	Unit	Reference and notes
Litter	Continuous	H_{max}	Height of the highest herbaceous species	%	Species < 5% of the total plot area were excluded
	Continuous	Dead	Ground cover of woody debris	%	
	Continuous	Wood	Ground cover of plant dead material	%	
C and N cycling	Continuous	OC	Topsoil (first 10 cm) organic carbon content	%	Samaritani et al. (2011) [25]
	Continuous	C	Topsoil (first 10 cm) total carbon content	%	
	Continuous	N	Topsoil (first 10 cm) total nitrogen content	%	
	Continuous	BR	Topsoil CO2 emissions (details in text)	ppm mg dry soil^{-1}	
Vegetation structure	Continuous	A_cov	Within plot percentage cover of the tree strata	%	
	Continuous	B_cov	Within plot percentage cover of the bush strata	%	
	Continuous	H_cov	Within plot percentage cover of the herbaceous strata	%	
	Continuous	Mosses	Within plot percentage cover of the mosses strata	%	
Flood dynamic	Continuous	Elevation	Elevation above sea level	m asl	
Soil morphology	Continuous	Large	>30 mm	%	Relative abundance of particles
	Continuous	Medium	10–30 mm	%	of given size in the topsoil (0–5 cm depth)
	Continuous	Small	5–10 mm	%	
	Continuous	Sand	1–5 mm	%	
FPZ	Binary	Pasture, Willow forest, Mixed forest, Forest, Willow Bush, Tall herbs		0/1	

diameter, width, and height) using a different formula for each test shape (Table 2):

$$\text{Hemisphere}: \ \text{Biovolume} = \text{Pi}*r^3*2/3 \quad (1)$$

$$\text{Saucer}-\text{shaped}: \ \text{Biovolume} = \text{Pi}/2*r^2*h \quad (2)$$

$$\text{Cylindrical}-\text{ovoid}: \ \text{Biovolume} = \text{Pi}/6*d^2*h \quad (3)$$

$$\text{Ovoid}: \ \text{Biovolume} = \text{Pi}/6L*w*h \quad (4)$$

Where r is the radius, h the height, d the diameter, L the length, and w the width of the shell.

Table 2
Summary of the trait values for each species.

	Phylogenetic grouping	Origin of the test material	Test shape	Position of the aperture	Test compression	Biovolume
Arc_dis	1	2	2	1	1	112,486
Arc_rot	1	2	2	1	1	28,599
Arc_vul	1	2	2	1	1	96,211
Ass_mus	0	3	4	2	1	12,370
Bul_ind	1	1	4	3	1	932,660
Cen_acu	1	1	4	3	1	73,304
Cen_acu_ob	1	1	4	3	1	411,275
Cen_aer	1	1	4	3	1	35,117
Cen_aer_sp	1	1	4	3	1	52,360
Cen_cas	1	1	4	3	1	101,137
Cen_con	1	1	4	3	1	237,583
Cen_eco	1	1	4	3	1	402,124
Cen_orb	1	1	4	3	1	527,788
Cyc_eur	1	1	2	1	0	42,379
Dif_obl	1	1	4	2	0	1,154,535
Dif_lin	1	1	4	2	0	65,424
Dif_mic	1	1	3	2	0	355,758
Dif_pen	1	1	4	2	0	36,757
Eug_lae	0	3	4	2	1	7257
Hel_pet	1	1	4	2	1	82,467
Phr_acr	1	1	1	1	0	23,856
Pla_cal	1	1	4	4	1	102,102
Pla_pen	1	1	4	4	1	66,183
Trigo_min	1	1	2	1	0	112,708
Trine_lin	0	3	3	3	0	6185

Species codes correspond to the three first letters of the genus and species name and, when necessary, the two first letters of the sub-species name. Phylogenetic grouping: 0 = Euglyphida, 1 = Arcellinida. Origin of the test material: 1 = Agglutinate, 2 = Proteinaceous, 3 = Siliceous. Test shape: 1 = Hemisphere, 2 = Saucer-shaped, 3 = Cylindrical-ovoid, 4 = Ovoid. Position of the aperture: 1 = Axial aperture, 2 = Acrostomic, 3 = Plagiostomic, 4 = Cryptostomic. Test compression: 0 = compressed, 1 = non-compressed. Biovolume is given in μm^3.

2.4. Numerical analyses

For each FPZ and for the entire restored area, we calculated the total number of species, the Shannon index [32], the species evenness, and the density (number of individuals per gram of soil dry weight). Four functional indices were also calculated in order to detect any changes in community functioning: functional richness (FRic), divergence (FDiv), evenness (FEve), and dispersion (FDis) [33,34]. FRic is the amount of niche space filled by species in the community calculated based on the convex-hull volume method [35]. Low values indicate that some resources available to the community are unused. FDiv measures the spread of abundance along a functional trait axis. High FDiv indicates a high degree of differentiation of the niche and a low competition for resources [36]. FEve accounts for the evenness of abundance distribution in a functional trait space. Low values show that some parts of niche space occupied are under-used [36]. FDis is a measure of multivariate dispersion that estimates the dispersion of species in trait space conceptually similar to Rao's quadratic entropy Q [37]. These indices represent different aspects of functional diversity and provide therefore complementary pieces of information that a single index could not account for. They were computed using the function "dbFD" of the *R* package "FD" [38]. Mann–Whitney tests were performed to assess differences among FPZs.

To assess whether trait convergence or divergence [39] may be discriminated in the different FPZs, a permutation test was computed. The latter consisted in permuting rows in the species per trait matrix (Q) to randomly attribute trait values to species and preserve species abundance and richness at the same time. FDis was preferred over the other indices [33] as test statistic and computed for each FPZ as well as for the restored area. This was repeated 1000 times allowing us to generate a probability

distribution (FDis_sim), which was used to calculate *p*-values. The three possible outcomes of this test are:

1) 5th percentile of FDis_sim < FDis < 95th percentile of FDis_sim => neither convergence, nor divergence of traits
2) FDis < 5th percentile of FDis_sim => convergence of traits
3) FDis > 95th percentile of FDis_sim => divergence of traits

Along the river perturbation gradient, we thus hypothesize that abiotic constraints will lead to convergence of traits (case 2) in the most dynamic FPZs while strong biotic interactions (competition) will lead to divergence of traits (case 3) in the most stable FPZs. In the intermediate situation neither convergence nor divergence should be observed (case 1).

To assess the relationships between species traits and environmental variables, we used the "Fourth Corner" statistic which measures the link between the species per traits (*Q*), the sites per species (*L*), and the sites per environmental variables (*R*) matrices [40,41]. To do so, we used the "fourth corner" function of the *R* package ADE-4 [42]. This function uses different types of correlation coefficients to measure the above-mentioned relationship, and test their significance through a permutation test. Environmental data were standardized prior to the analyses and 1000 repetitions of row permutations in *L* were computed. This procedure allows preserving the relations between *L* and *Q* and corresponds to permutation model two of Dray and Legendre [41]. The *R* matrix consisted in the quantitative data (% cover of vegetation, ground cover, particle size distribution in the topsoil, physico-chemical soil variables, soil respiration, and plot elevation) and five dummy variables constructed to represent the six different FPZs. *P*-values were adjusted using Holm's correction to avoid increases of type error I due to multiple testing [43]. All analyses were conducted with the *R* software for statistical computing [44].

3. Results

3.1. Testate amoeba diversity and functional indices

In total, 25 TA species were identified. *Centropyxis* and *Difflugia* were the more common genera. The most common species for both areas was *Plagiopyxis penardi*. Three species were restricted to the reference area, whereas seven occurred only in the restored area (Table 3). The density of *Arcella discoides* was 87% lower, and *Difflugia penardi* density 213% higher in the restored area when compared to Pasture. All other species that occurred in both areas showed smaller relative differences.

Average TA species richness, was lower in the restored FPZs than in the reference site (respectively 5.9 and 9.8, $P = 0.01$). Similarly, diversity was lower on average in the restored FPZs than in the reference site (respectively 1.6 and 2.1, $P = 0.01$). There was no significant difference in density between the restored area (1072 ± 121 ind. g soil^{-1}) and the reference area (1039 ± 124 ind. g soil^{-1}). Functional richness, evenness, diversity, and dispersion were lower in the restored area (0.26, 0.49, 0.72, and 1.01 respectively) than in the reference site (0.41, 0.51, 0.73, and 1.25 respectively), but not significantly ($P > 0.05$).

Clearer differences among FPZs were observed for species richness, species diversity, density and species evenness as compared to functional indices (Fig. 1). Pasture stood out by high species richness and diversity and lower species evenness. Species richness and diversity were low in Willow forest. Tall herbs stood out by low density but relatively high species richness, diversity and evenness. There was no significant difference among the three intermediate FPZ (Willow bush, Forest and Mixed forest) for species richness, species diversity, density or species evenness. No difference was found among FPZs for functional divergence. Functional richness was higher in Willow bush and Pasture than in Willow forest (both $P = 0.05$). Functional dispersion was higher in Willow bush than in Forest and Willow forest ($P = 0.05$ and 0.03 respectively). Functional evenness was higher in Tall herbs than in Forest and Mixed Forest ($P = 0.05$ and 0.03 respectively).

Table 3
Average density [individuals g soil^{-1}] of each species of testate amoeba in the restored and reference areas.

	Restored	Reference
Arcella discoides	3.0	22.6
A. rotundata stenostoma	0.0	22.6
Arcella vulgaris	0.0	11.3
Assulina muscorum	45.9	56.5
Bulinularia indica	10.5	45.2
Centropyxis aculeata	18.8	11.3
C. aculeata oblonga	6.8	0.0
C. aerophila	17.3	56.5
C. a. sphagnicola	112.9	90.3
C. cassis	85.8	56.5
C. constricta	76.8	56.5
C. ecornis	2.3	0.0
C. orbicularis	10.5	56.5
Cyclopyxis eurystoma	4.5	0.0
Difflugia oblonga	14.3	0.0
D. lineare	6.0	0.0
D. microstoma	6.0	0.0
D. penardi	70.8	22.6
Euglypha laevis	0.0	11.3
Heleopera petricola	25.6	67.8
Phryganella acropodia	68.7	45.2
Plagiopyxis callida	141.2	112.9
P. penardi	333.2	271.0
Trigonopyxis minuta	10.5	0.0
Trinema lineare truncatum	0.0	22.6

3.2. Traits convergence and relationships to environmental variables

The permutation tests suggest that all FPZs correspond to case 2 (convergence of traits, all $P < 0.01$). In the fourth-corner analysis, litter decomposition, vegetation structure, and the type of FPZ all had at least one trait significantly correlated with one of their representative variables. None of the measured traits were significantly related to flood dynamic, soil morphology, and C and N cycling. With the exception of siliceous tests all species traits were correlated to at least one environmental variable (Fig. 2).

Our analyses revealed a positive relation between shell biovolume and the relative cover of mosses and a negative correlation with the Willow forest FPZ. We found a negative relation between shell compression and the cover of plant litter (both dead wood and non-woody) and a positive one with the relative cover of herbaceous vegetation. The origin of the test material showed similar relations with the relative cover of herbaceous vegetation and the cover of non-woody plant litter. A negative relation was found between Aperture Position and both Tall herbs and Willow forest. Finally, we obtained a relation between Phylogenetic grouping and the relative cover of non-woody plant litter on the ground.

4. Discussion

4.1. Testate amoeba: community patterns and functional traits distribution

This study revealed contrasted patterns of TA density, diversity and functional traits in the restored and control sites of the River Thur floodplain. Density, species richness and diversity were all low

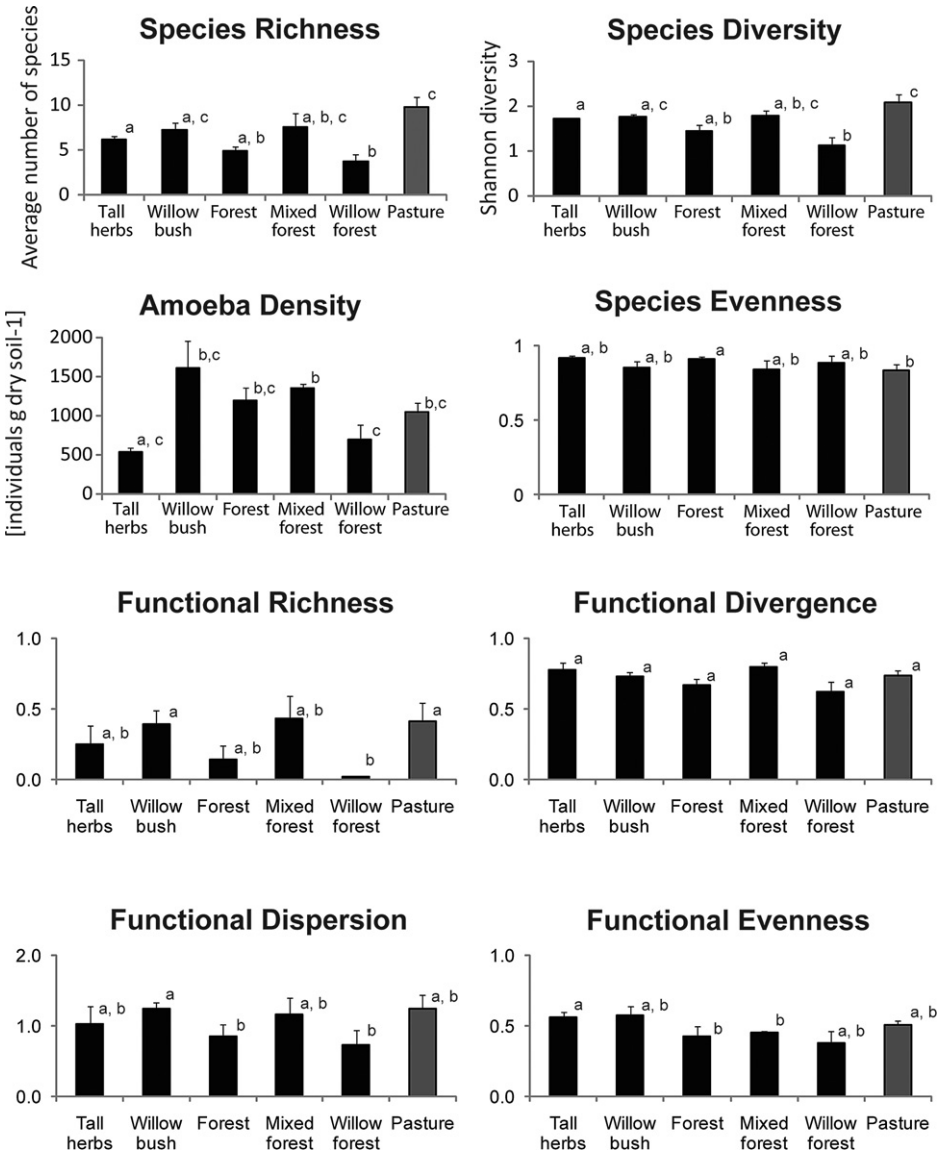

Fig. 1. Testate Amoebae average species richness, Shannon diversity, density, and species evenness, and functional richness, evenness, divergences, and dispersion for each functional process zone (FPZ). Standard errors are represented. Black bars indicate FPZs from the restored area, whereas grey bars indicate the reference FPZ (Pasture). Different letters indicate significant differences of the means (Mann–Whitney tests).

by comparison with more favourable habitats such as upland forest soils [9]. There was no overall difference in density as differences among FPZs of the restored area balanced each other. In agreement with the supposed lower affinity of TA for early succession habitats [45] density was lowest in the most dynamic FPZ. In agreement with the general positive relationship between habitat diversity and species richness [46], TA total species richness was higher in the restored area than in the reference site and both species richness and Shannon diversity were lower in individual FPZs of the restored area than in the reference area (significantly for three out of five FPZs).

Although the restored area covers a wider range of FPZs than the reference site and clear differences among FPZs were identified using classical indices, none of the four functional diversity indices differed between the two areas. This suggests that functional and classical indices provide complementary insights on the structure and functioning of ecosystems.

However, differences among FPZs along the gradient were identified for all functional indices except for Functional Divergence. These are interpreted here in agreement with Mason et al. [36]. Functional Divergence was relatively high for all FPZs indicating a low competition for resources. Functional Richness was generally low, and especially so in Willow forest, indicating that resources were either scarce or poorly exploited. At the site level, in all FPZs, low Functional Richness indicates that TA communities do not reach equilibrium and do not optimally exploit resources. Low Functional Richness indicates the existence environmental pressure, in this case the most likely due to flood dynamic-related factors. As a result, the importance of competition in shaping communities is low. This interpretation is in line with the observation of absence of replacement in TA community assembly in a primary succession [47]. At the level of individual sites, the available niche space is occupied to a greater extent (i.e. the within site distribution of biomass-weighted relative proportion of different TA FT in the available niche space is more uniform) close to the river than in the forest, as indicated by the higher Functional Evenness.

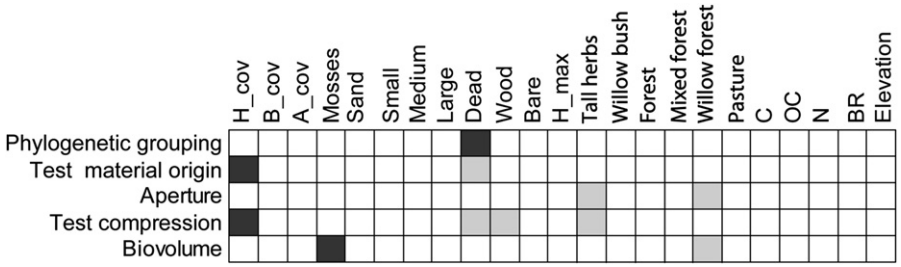

Fig. 2. Relationships between environmental variables and testate amoeba functional traits. Only significant correlations are indicated ($P < 0.05$). Dark grey indicates positive associations, and light grey negative ones.

4.2. Functional dispersion: convergence of traits?

We hypothesized that trait convergence would be highest in the most dynamic FPZs and would decrease towards more stable FPZs. In disagreement with our hypothesis, traits were convergent in all FPZs. This shows that environmental filters are selecting TA FT and suggests that these filters were not directly related to flooding, or that alternative, complementary filters were acting in different FPZs (e.g. grazing pressure in the pasture, waterlogging in the Willow forest). Flood-related effects on biotic communities are well-known for higher plants [48] and animals [49,50], but our result tend to show that filters are different for protists than for plants and arthropods. Defining and testing the effects of these filters will improve our understanding of soil microbial ecology and requires further studies ideally combining descriptive approaches such as used here (to confirm the observed pattern for TA and/or other soil organisms) and manipulative experiments (to test the effect of specific factors).

The convergence of traits identified here may depend on the set of traits we selected initially. Results may differ for set of traits specifically related to dispersal or reproduction for example. The approach presented here shall therefore be considered as a first step toward the selection of relevant functional traits for TA ecology. The next step was to assess the relationships between FT and environmental variables.

4.3. Relationships between species traits and environmental variables

Trait convergence implies that strong environmental filters are forcing species assembly patterns. The different responses of functional indices along environmental gradients agree with the idea of high functional redundancy in soils [51] and can be explained by the relationships among environmental variables and species traits. These relationships are either direct functional relationships or indirect trait-adaptation to habitat.

As aquatic soil organisms, TA live in water films and are particularly abundant in soils with organic humus, high moss cover, and generally sufficiently frequent moist conditions such as peatlands, forests [52]. Soils with a well-developed humus have a higher water holding capacity [53] and generally contain a high density of TA [9]. Such conditions are expected to favour species with uncompressed shells, unprotected aperture, and/or large biovolume. Soil moisture content and dynamics therefore control the density and also community structure of TA.

However TA are believed not to tolerate anoxia. As a result, certain species may regularly die out in waterlogged soil patches and subsequently re-colonize them from adjacent areas. In this case, smaller species will be favoured because they are more likely to be dispersed passively over long distances (owing both to potentially larger population size and small size) [54]. Certain species may encyst and enter a latent phase to cope with anoxia. This strategy may be favoured by larger species with relatively low dispersal ability.

Favourable soil moisture conditions can result from different factors such as high water table, thick litter or moss layers, and shading by trees, etc. Our results show a complex pattern of correlations between individual habitat variables that influence soil moisture and TA species traits. These results add to the well-documented correlation between TA communities and soil moisture variables [55].

In this study we did not address the vertical distribution of TA along the soil profile nor the seasonal variations. Given how dynamic floodplain ecosystems are it would be useful to address these questions. The results we obtained are promising, but further comparable descriptive studies are required to provide true replication. In addition, manipulative experiments should be conducted to address specific questions such as long-term and cyclical effect of flood duration, intensity, and frequency on TA communities.

Finally the set of traits and the environmental variables considered in this study are in no case exhaustive. The selection of relevant functional traits is a critical point for such approaches [56]. With this respect, traits related to the test (i.e. Biovolume, Shell Compression, Aperture, and Test Material Origin) were strongly associated to litter variables and constitute therefore the best candidates for bioindication. It is premature to propose an index based on these traits but our results show that this can be achieved. The relationship between Phylogenetic Grouping and woody debris is difficult to explain and requires further research to assess its potential value for bioindication. We encourage future studies to develop similar approaches of soil microbial ecology and study additional traits (e.g. related to the cyst forming capacity of TA that may determine their capacity to withstand periods of unfavourable conditions including anoxia).

5. Conclusion

This work confirmed that environmental filters are forcing the assembly patterns of TA communities in a restored floodplain and demonstrated the strong relationships between environmental variables related to soil moisture and TA species traits.

In agreement with the idea of functional redundancy among soil organisms, the response of TA to perturbations was clearer for density and diversity than for FT. Selection of FT is however a critical step. We show clear response for shell-related traits but responses of other, e.g. physiological traits such as encystment capacity, should be explored.

Finally, the spatial and temporal complexity of floodplain ecosystems represents an ideal setting to study the factors controlling the distribution of soil micro-organism and their associated functional traits along environmental gradients. The complexity of the system however calls for combined descriptive and experimental studies.

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