Standard Paper

Plant functional diversity drives niche-size structure of dominant microbial consumers along a poor to extremely rich fen gradient

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Running title: Plant types key to microbial consumers in fens

Summary

1. Plant community composition is more and more recognized to play an important role on ecosystem processes, such as C cycling. Especially plant functional type (PFT) composition seems to have a key regulatory role, yet the underlying mechanisms in the interaction between PFTs and ecosystem processes are still to be identified.
2. Here, we assess the link between PFTs and dominant microbial consumers along a calcareous poor- to extremely rich-fen gradient in western Poland. We particularly focussed on dominant microbial consumers (testate amoebae), which can exert large effects on the functioning of peatlands. Using moving-window analyses and path-relation networks subjected to structural equation modelling (SEM), we investigated linkages among abiotic factors, PFTs and testate amoebae.
3. We show that along the poor- to extremely rich fen gradient the dependence of testate amoebae to PFTs is higher than their dependence to abiotic factors. We also found that the link between testate amoebae and PFTs differs between size assemblages of testate amoebae. While large testate amoeba species (i.e. high trophic level) were highly linked to *Sphagnum* mosses cover, small species (i.e. low trophic level) were linked to brown mosses. Distinction between shallow-rooted and deep-rooted vascular plants also showed that shallow-rooted plants play a role on testate amoeba community structure at the ‘poor’ side of the gradient.
4. Our results further show a dominant role for calcium content and the structure of the bryophyte community on testate amoeba size-assemblages at the poor- to extremely rich fen scale, both for diversity and abundance of testate amoebae.
5. *Synthesis*. Variations in plant functional type composition drives niche-size structure of testate amoebae along the (calcareous) poor- to extremely rich-fen gradient. Furthermore, strong relationships between moss types and testate amoeba size structure suggest that mosses specifically influence testate amoeba development through autogenic effects. Therefore, moss cover composition is key to microbial consumers and may be the driving factor determining microbial network structure and associated ecosystem processes, such as carbon cycling.

Key-words: bryophytes, functional types, functional traits, peatland, plant-soil (belowground) interactions, soil organisms, testate amoebae, size-structure

Introduction

Understanding the interactions between above- and belowground components in driving community and ecosystem properties is increasingly recognized as a fundamental element of ecosystem ecology and functioning (Bardgett & Wardle 2010). Particularly, the link between plant and soil microbial communities is key to ecological processes, such as carbon and nutrient cycling (Wardle *et al.* 2004). Plants, by their exudates and organic matter production, are critical in regulating belowground communities and associated biogeochemical cycles (Wardle *et al.* 2012). On their turn, belowground communities drive decomposition and nutrient mineralization, and are as such important for the ecosystem nutritional status (Schimel & Bennett 2004; Jones *et al.* 2005), and ultimately the plant community composition. While a rapidly growing number of studies report above- and belowground communities to interact directly and indirectly across different levels of ecological organization (Wardle *et al.* 2012), the identification of such linkages in peatlands still remains understudied (Ward *et al.* 2009; Jassey *et al.* 2013). Recent work has shown that divergent plant communities modify the robustness of greenhouse gas fluxes in peatlands (Ward *et al.* 2013; Kuiper *et al.* 2014), but their impact on belowground communities and processes is yet to be understood.

To bridge this knowledge gap, we established a gradient study aiming to examine the relationships between plant and microbial consumer communities in fens—organic rich and waterlogged ecosystems. Fens are diverse, nutrient-poor, and groundwater dependent systems, harbouring many globally endangered plant species and communities (Wassen *et al.* 2005). They can be divided into poor- and rich-fen, even sometimes with a finer grouping into extremely poor, poor, intermediate, rich and extremely rich (Rydin & Jeglum 2006). Poor fens are oligotrophic and slightly acid (pH < 6), while rich fens are minerotrophic, enriched in calcium and a pH between 6 and 8 (Sjörs & Gunnarsson 2002). These abiotic factors, as well as water table depth, strongly influence the plant community structure in fens (Lamentowicz *et al.* 2010). Several studies report changes in plant community composition along poor to rich fen gradients (Wheeler & Proctor 2000; Hájek *et al.* 2006; Sekulová, Hájek & Syrovátka 2013). Generally, brown-moss abundance increases in calcareous rich-fens, with a parallel decrease in *Sphagnum* abundance (Vitt *et al.* 1995; Hájek *et al.* 2006). Vitt and

Chee (1990) demonstrated that bryophytes respond to gradients in acidity and alkalinity, while vascular plant patterns are more responsive to nutrient gradients, especially nitrogen and phosphorous. These divergent responses to environmental conditions between typical plant functional groups complicate analyses on the functional response of peatlands to such changes. Additionally, despite recent comprehensive studies on understanding plant diversity and patterns of distribution along ecological gradients (Vitt *et al.* 1995; Hájková & Hájek 2003), data on the interactions between plants, mosses and microorganisms are very scarce. In fen-bog gradients it has been demonstrated that there is a clear link between the plant community composition and the microbial community composition (Lamentowicz *et al.* 2010). However, an important gap remains considering the true effect of plant functional types on soil biota community structure in fens. Identifying and understanding such interactions is fundamental for predicting future changes in above- and belowground community composition and ecosystem function in fen ecosystems. As above- and belowground linkages tightly regulate biogeochemical cycling and potential climate feedback in peatlands (Jassey *et al.* 2013; Lamentowicz *et al.* 2013), and given that fens are key ecosystems owing to their ability to store large amounts of carbon (Vitt *et al.* 2009), such knowledge is all the more important.

Here we study how and to what extent plant functional types (*Sphagnum* and brown mosses, and vascular plants) determine soil microbial assemblages (specifically the protozoa, testate amoebae) along a poor- to rich-fen gradient. Because of the strong variations in abiotic factors among fen types (e.g. pH, water table, and nutrient status), we hypothesize that (i) patterns of species richness and diversity for plants and testate amoebae follow similar trends along the gradient and (ii) such changes are mainly driven by pH and base richness (calcium or conductivity). We predict that (iii) abiotic factors have less influence on testate amoeba community composition than plant functional types over the gradient. Indeed, the correlation between abiotic fen types properties and testate amoebae is expected to decline along the gradient because of the successive assembly patterns of plants. As the nutrient status of fens increases between poor and rich fens, vegetation cover and species turnover increase (Vitt *et al.* 1995; Hájková & Hájek 2003). Therefore, plant-microbial interactions should increase along the gradient, thereby reducing the relative influence of abiotic factors on testate amoebae. As dominant microbial consumers in the microbial food web, testate amoebae represent an important trophic link between organic matter, decomposers and plants (Ledeganck, Nijs & Beyens 2003), ~~. ~~w~~W~~e finally predict (iv) that patterns of testate amoebae (diversity and community composition) and especially their trophic structure—assessed using their functional traits (e.g. body size) (Jassey *et al.* 2013)—follow the trophic status of fens, and thus the patterns of plant functional types along the gradient.

Materials and Methods

STUDY SITES

Study sites were located in western Poland (Wielkopolska region) (Fig. 1a, Table 1), and represent various trophic states and vegetation types (Supplementary Table 1). We selected eight fens from this region because they were only slightly affected by human and because they are close to pristine state. Also, the sites are representative for the young glacial landscape of Central-Eastern Europe. Because of their unique character, several fens such as Rurzyca, Kuźnik Bagienny, Wierzchołek, and Czarne are protected in the Natura 2000 framework. Sedges and rushes such as *Schoenoplectus tabernaemontani*, *Cladium mariscus and Carex rostrata* mainly dominated vascular plant communities in these fens. *Oxycoccus palustris*, *Eriophorum angustifolium*, *Carex rostrata*, and *Eleocharis quinqueflora* characterize poor and moderately rich fens, while *Menyanthes trifoliata* and *C. diandra* characterize rich fens. Extremely rich fens differed from rich fens with the dominance of *Thelypteris palustris* and *Schoenoplectus tabernaemontani*. As vascular plant communities, bryophytes show different patterns along the gradient. *Sphagnum* species such as *S. fallax* and *S. angustifolium* characterize poor and moderately rich fens, as well as acidic microhabitats in extremely rich fens. Brown mosses such as *Calliergonella cuspidata and C. giganteum* dominated rich to extremely rich fens. More details on dominant vegetation per plot can be found in Supplementary Table 1.

Sampling plots in fens were chosen to cover the widest spectrum of microhabitats available in each fen, and thus capture as much as possible the plant and testate amoeba diversity in each fen. A total of 132 plots were selected in eight fens (Table 1) and sampled during the vegetative seasons (July—August) in 2008 and 2009. Several microhabitats—including floating-mat margins, hollows, lawns, and hummocks (Table 1)—were distinguished in each fen. These were further separated based on vegetative features (e.g. moss cover, moss type (*Sphagnum* and brown-mosses) and dominant vascular plants). As such, each site differed based on the presence of these microhabitats and vegetative features (Table 1).

Vegetation cover (%) was recorded (1 × 1 m) for each plot, and later transformed according to the Van der Maarel scale (Van der Maarel, 1979). Vascular plant nomenclature follows Mirek *et al.* (2002); that for bryophytes Ochyra *et al.* (2003). Vascular plants and mosses were divided into plant types—based on our own knowledge and field observations: shallow-rooted (0-20 cm deep, e.g. *Oxycoccus palustris*, *Drosera rotundifolia*) and deep-rooted (> 20 cm deep, e.g. *Carex rostrata*, *Eriophorum angustifolium*) vascular plants, and *Sphagnum* and brown mosses, respectively (Supplementary Table 1). Additionally, in all plots a sample was collected to determine the testate amoebae community.

TESTATE AMOEBA ANALYSES

Peat soil samples, including living parts of the mosses (brown mosses and *Sphagnum*) and fine roots of vascular plants, were cut with a serrated knife and then were placed in plastic containers (diameter 6 cm; depth 8 cm). In the laboratory, testate amoebae were extracted from the samples immediately after sampling by washing with water through 20 and 300-μm mesh sieves and transferred into glycerol storage medium (Booth, Lamentowicz & Charman 2010). The resulting ‘testate amoeba’ samples were then stored at 4˚C until further analysis. Testate amoebae were identified and counted at ×200 and ×400 magnification using light microscopy, to reach a total of 150 shells (see Payne & Mitchell 2009). Identification at species level was based on available literature (Ogden & Fairman 1979; Ogden & Hedley 1980; Clarke 2003; Mazei & Tsyganov 2006).

Recent studies have shown that testate amoebae can be separated into two main groups, large vs. small taxa, based on their foraging traits such as diameter of shell-aperture size and body size traits (Jassey *et al.* 2013; Lamentowicz *et al.* 2013). These functional traits reflect their trophic position within the food web, high and low, respectively. Species with a small body size (< 60 µm) and small shell aperture diameter (<15 µm) are usually grazers (i.e. species with a low trophic level) feeding on bacteria and fungi, while species with a large body size (> 60 µm) and large shell aperture diameter (> 15 µm) are usually primarily predators (i.e. species with a high trophic level) feeding on larger prey such as other protists, rotifers and nematodes (Wilkinson & Mitchell 2010). Threshold values of body size and shell aperture diameter for small and large taxa were based on the frequency and range of these two traits within the community (see Supplementary Figure 1) and our personal observations of feeding habits of testate amoebae. Using these traits, we assessed the distributional patterns of small *vs.* large testate amoebae across fens. Values of body size and shell aperture diameter were assessed using microscopy and image analyses. Details on testate amoeba species density and trait values are given in Supplementary Table 2 and 3.

CLASSIFCATIONS OF FENS IN NUTRIENT AVAILABILITY CLASSES

Water table depth (DWT), groundwater pH, and conductivity (EC) were measured directly in the field using a centimeter measure and portable multimeter Elmetron CX742, respectively. Three ground-water subsamples (1.5 dm^3) were collected from each plot and stored in plastic bottles. The first bottle contained 1 ml of concentrated nitric acid, the second contained 1 ml of 95% chloroform, while the third bottle served as a reference (i.e. no addition of preservation chemicals). The variables measured were: NH_4^+ (by Nessler's colorimetric method), NO_3^- (by the cadmium reduction method), PO_4 (by the colorimetric ascorbic acid method), SO_4 (by the nephroometric method), Ca^{2+}, Mg^{2+}, K^+, Na^+, and Fe^{2+} (atomic absorption spectrometry method).

Cluster analysis (Ward method) on the physicochemical properties of the abovementioned water samples was used to *a posteriori* divide the fen sites into four types (Fig. 1b): poor fens (PF), moderately rich fens (MRF), rich fens (RF) and extremely rich fens (ERF). Kuźnik Bagienny (KB) appeared to be the only poor fen in our data set. We found that increased Ca^{2+} concentrations drove such classification, while factors such as phosphates, sulphates, nitrates, and pH, thought important, played a lesser role (Fig. 1a, Supplementary Table 4). Usually, pH is the single factor categorising fen types (Hájek *et al.* 2006), but our results showed that fen types distinction is more precise using Ca^{2+}. With 7 mg L^{-1} the poor-fen had lowest level of calcium. Moderately rich fens contained on average 49.2 mg Ca^{2+} L^{-1} while rich fens contained on average 90.5 mg Ca^{2+} L^{-1}. Kuźnik Olsowy (KO) and Makaty (MAK) represent moderately rich fens, while Wagowo (WAG), Rurzyca (RURZ) and Wierzchołek (WEK) represent rich fens. Kazanie (KAZ) and Czarne (CZAR) represent extremely rich fens and the highest amount of dissolved Ca^{2+} ranged between 125 and 155 mg L^{-1}. All of the investigated fens were nutrient poor, although significant variations were found among fen types (Supplementary Table 4).

NUMERICAL ANALYSES

Gradient analysis has two spatial structures: spatial processes within the community, which create autocorrelation, and the spatial structure in environmental factors, which in turn causes spatial dependence in biotic communities (Wagner 2004). We assessed and tested spatial autocorrelation for moss, vascular plant and testate amoeba communities by means of Mantel correlograms (Borcard, Gillet & Legendre 2011). Then, we tested spatial dependence in the three communities by means of direct multiscale ordination (MSO), which combines multivariate ordination analyses and geostatistics (Wagner 2004). The ‘grain’ (i.e. interval size for distance classes) of the variogram was chosen ~~to be~~as the truncation threshold from a Principal Coordinates of Neighbourhood Matrix (PCNM) analysis applied on the fen coordinates ~~of fens~~ (Borcard *et al.* 2011). The truncation threshold value corresponds to the longest distance in the minimum spanning tree of the spatial matrix. In none of the three communities species compositions were spatially autocorrelated, except for closer sites such as Kuźnik Bagienny and Kužnik Olsowy (Fig. 1), and only for moss and vascular plant communities (Supplementary Figure 2). Thus, there is any confusion between biotic community structure across fens and spatial processes internal to the community itself. Similarly, MSO showed that species-environment relationships are scale invariant (Supplementary Fig. 3), indicating a true gradients in species composition driven by the environmental gradient (Legendre 1993).

All community data sets were Hellinger-transformed (Legendre & Gallagher 2001), after which we used redundancy analyses (RDA) to relate abiotic factors and biotic communities (mosses, vascular plants and testate amoebae). Since plant communities showed significant spatial trends, we spatially detrended RDAs on plant communities by using partial RDAs with location factors as co-variables. Stepwise selection of explanatory variables among the whole set of abiotic factors was computed for each biotic community using the ‘ordistep’ function available in the *vegan* R package (Oksanen 2011). This stepwise selection modelling was automatically achieved using AIC (Akaike Information Criterion) and permutation tests.

Linear mixed effects models were used to determine whether species richness, diversity and cover (or relative abundance) of vascular plants, bryophytes and testate amoebae vary among fen types (fixed effect) while accounting for the repeated measurements within each site. All models included site as a random effect on the intercept, as to correct for the inflation of the residual degrees of freedom that would occur if we were using repeated measurements within sites as true replicates (Pinheiro & Bates 2000). The interaction between spatial coordinates of fens and fen types was also tested in all models to determine whether richness, diversity and cover (or abundance) of our biotic communities vary according to spatial coordinates or fen types. No significant interactions were found, showing that spatial coordinates of fen did not affect these parameters. Post-hoc analyses were then used to determine the differences and/or similarities among fen types. The same analyses were performed on shallow and deep-rooted vascular plants; brown and *Sphagnum* mosses; and small and large testate amoebae. We also computed spearman’s rank correlation tests between richness and diversity indices to assess monotonic relationships between biotic communities. Further, w~~W~~e ~~also~~ tested the effect of plant cover (vascular plants and/or bryophytes) and several abiotic factors on the relative abundance of testate amoebae using linear mixed effects models, as specified above. These tests were computed on non-spatially detrended data.

To evaluate changes in the relationships between testate amoebae and plant functional types across the gradient, we used moving-window analysis (Carlson *et al.* 2010). This technique allows the analysis of multivariate data across an ecological gradient and is particularly useful for the detection of sharp transitions in species composition across gradients (Kent *et al.* 1997). This analysis was computed using a window of 70 consecutive samples (multiple window sizes were tested initially, all showing similar patterns; the 70 sample window size was, however, optimal for interpretation without losing detail on the studied relationships) across sites to quantify and to test the variation of linkages among the three biotic communities and abiotic factors over the gradient. The 70-sample window was advanced across the fen gradient sampling plot by sampling plot (e.g. 1-70, 2-71, 3-72…62-132) after reordering the matrices following the calcium gradient. We used redundancy analyses, adjusted R^2 and permutation tests to assess the relationships (Oksanen 2011). The ten Hellinger-transformed community matrices (total plants, mosses, vascular plants, brown mosses, *Sphagnum* mosses, shallow-rooted and deep-rooted vascular plants, testate amoebae, and large and small testate amoebae) were used as response matrices. Due to unbalanced matrices, direct comparison of the species matrices was not possible. Therefore, from all species matrices we extracted the scores of the first three PCA axes. As such an indirect comparison between the plant and microbial matrices could be made (Carlson *et al.* 2010).

We considered all available variables to investigate global linkages between environmental factors, plant functional types and testate amoebae. Using *a priori* knowledge, a full model of causal relationships was created including simultaneously several hypothetical pathways (Table 2) through which the gradient—characterized by calcium content in water—could affect plant and testate amoeba communities (Supplementary Figure 4). The full model included a direct pathway between calcium and plant and testate amoeba community composition/abundance/diversity, and several indirect pathways via plant-microbial relationships (See Supplementary Figure 4). Then, successive full models were simplified by step-wise exclusion of variables with non-significant weights and non-significant covariance, as estimated by AIC, until a minimal adequate model showing specific linkages remained. Principal component analyses (PCA) were computed on Hellinger-transformed plant functional type and testate amoeba data sets to extract community composition indicators along the gradient. The first three axes of each PCA were tested in the SEM models and selected by step-wise selection using AIC.

Because water chemistry can directly influence plants and testate amoebae, we purged biotic factors from the effect of water chemistry prior to modelling specific interactions between plants and testate amoebae in SEMs. This was accomplished by fitting successive linear mixed effect models testing the effect of calcium on response variables used in SEM model, and saving the residuals from each linear mixed effect models as new variable. Because of this step, variation in biotic factors caused by environmental factors cannot influence the outcome interactions between plants and testate amoebae in SEMs. The adequacy of the models was determined using χ^2 tests, Standardized Root Mean Square Residual index (SRMR), Root Mean Square Error of Approximation index (RMSEA), Akaike value (AIC), and Comparative Fit Index (CFI). Adequate model fits are indicated by non-significant differences when comparing the predicted and observed correlation matrices (χ^2 tests with $P > 0.05$), by lower AIC, RMSEA < 0.05, SRMR < 0.05, and CFI > 0.95 (Grace *et al.* 2010).

All computations were performed in R 3.0.1 (R Development Core Team, 2013) using the *vegan* (Oksanen 2011), *nlme* (Pinheiro & Bates 2000), and *sem* (Fox 2006) packages.

Results

RELATIONSHIPS BETWEEN BIOTIC COMMUNITIES AND FEN ABIOTIC VARIABLES

The separate RDAs show somewhat contrasting species-environment relationships for the three communities (Fig. 2). For mosses, vascular plants and testate amoebae, the best models selected four explanatory variables, which together explained 11% (mosses), 12% (vascular plants) and 26% (testate amoebae) of the variance (adjusted R^2). The three communities were all related to pH, calcium and sulphate content. In addition, mosses were ~~also~~ related to nitrates; vascular plants to Fe; and testate amoebae to DWT, respectively.

The three communities also differ with respect to the relative position of sites in the ordination space. In all analyses, the first two axes were significant ($P < 0.001$). For mosses, two looser groups emerged from the first axis (Fig. 2a): one group composed of PF and MRF sites and another one composed of RF and ERF sites. These two groups are mainly characterized by the presence (PF/MRF) and the near absence (RF/ERF) of *Sphagnum* mosses, respectively. For vascular plants, similar patterns emerged on the first axis, although the difference between PF/MRF and RF/ERF was better defined than for mosses (Fig. 2b). The group RF/ERF could be further split into RF and ERF on the second axis. Higher concentrations in Fe in rich fens underline this difference. For testate amoebae (Fig. 2c), three groups emerged from the first axis: one well-defined group mainly composed of PF sites and some sites from RF and ERF, a second looser group composed of MRF sites and a third one, well-defined, composed of RF and ERF sites. This last group could again be further split into RF and ERF on the second axis of the ordination space due to drier conditions in ERF sites (Fig. 2c, Supplementary Table 4). In all analyses, higher values of calcium, sulphates and pH explained the difference between PF/MRF and RF/ERF.

PATTERNS IN SPECIES RICHNESS, DIVERSITY AND DENSITY OF BRYOPHYTES, VASCULAR PLANTS AND TESTATE AMOEBAE AMONG FEN TYPES

We identified 38 bryophyte species, 80 vascular plant species, and 141 taxa of testate amoebae along the poor to extremely rich fen gradient. Species richness and diversity of the three communities (mosses, vascular plants, and testate amoebae) differed significantly along the gradient (Fig. 3). Vascular plant and moss species richness was highest in MRF (Fig. 3a, b, d, e). Separation of the bryophytes into *Sphagnum* and brown moss functional types showed slightly different patterns of richness and diversity. MRF and RF had highest brown moss diversity and richness (Fig. 3a, b), while *Sphagnum* richness and diversity were highest in MRF only (Fig. 3a, b). A significant negative monotonic relationship was found between species richness of brown and *Sphagnum* mosses (Table 3).

Fen type differences in richness and diversity were also found among vascular plant functional types. Richness and diversity of deep-rooted (graminoid) vascular plants did not vary along the gradient, while richness and diversity of shallow-rooted (ericoid) vascular plants was lowest in PF (Fig. 3d, e). No significant monotonic relationship was found between species richness and diversity of deep-rooted and shallow-rooted vascular plants, but a positive relationship existed between brown mosses and non-graminoid vascular plants (Table 3).

Testate amoebae richness and diversity increased over the gradient (Fig. 3g, h). Size split within the community showed that small testate amoeba species richness and diversity did not vary along the gradient, whereas these metrics were lowest in PF for large species (Fig. 3g, h). The monotonic relationship between small and large testate amoebae was significant for richness and diversity (Table 3). We also found significant monotonic relationships between large testate amoebae and brown mosses, as well as between small testate amoebae and brown and *Sphagnum* mosses (Table 3).

Total density of *Sphagnum* mosses gradually decreased over the PF to ERF gradient, whereas density of brown mosses was highest at MRF and RF (Fig. 3c). Total density of deep-rooted vascular plants did not vary along the gradient, while the density of shallow-rooted vascular plants decreased from PF over the gradient (MRF/RF/ERF) (Fig. 3e). Relative abundance of large testate amoebae followed patterns of *Sphagnum* mosses with a gradual decrease from PF to ERF (-45%; Fig. 3i). Similar tendencies were found with calcium content, which significantly increased from PF to ERF (Supplementary Table 4). Analysis of variances on spatially detrended factors showed that *Sphagnum* cover explained 22% of the variations of testate amoebae relative abundance (ANOVA, $P < 0.001$), while calcium explained 12% (ANOVA, $P < 0.01$).

UNDERLYING CAUSAL RELATIONSHIPS BETWEEN TESTATE AMOEBA AND PLANT FUNCTIONAL TYPES ALONG THE POOR-RICH FEN GRADIENT

Moving window analyses show that the dependence of testate amoebae to abiotic fen conditions decline after poor- and moderately rich-fen stages (i.e. further along the gradient), while relationships between plant communities and abiotic fen conditions remain stable or slightly increase along the gradient (Fig. 4a). In parallel, moving window analyses show that the dependence of testate amoebae to plants remains high and stable over the gradient; even it differs between mosses and vascular plants (Fig. 4b). The dependence of testate amoebae to mosses shows an inverse bell-shape response, peaking across the mid-successional stages of fens, while a decline between vascular plants and testate amoebae is evidenced. Detailed analysis of the relationships among size-types of testate amoebae and plant functional types showed that the dependence of small testate amoebae to *Sphagnum* mosses was maximal halfway of the gradient (on average 0.25; Fig. 4c), while for large species it decreased from 0.20 and 0.10 (Fig. 4c). Furthermore, the dependence of small and large testate amoebae to brown mosses was overall higher than with *Sphagnum* mosses (Fig. 4c), although it strongly decreased for larger specimens at the end of the gradient. The dependence of small testate amoebae to shallow- and deep-rooted vascular plants tended to be stable over the gradient, while it decreased for large testate amoebae. The relationships were the highest with shallow-rooted vascular plants for both size-types of testate amoebae (Fig. 4d).

The fit of all minimal adequate path analysis models was good (Supplementary Table 5). SEMs show clear causal linkages between PFTs and testate amoeba functional sizes over the gradient (Fig. 5). SEMs based on vegetation composition showed that calcium highly explained plant community structure, especially the community composition of mosses (Fig. 5a). The lack of direct paths between plant diversity and calcium indicates that calcium is less important predictor for overall plant diversity (Fig. 5b). Detailed examinations of PFTs showed that calcium did not significantly drive the abundance of shallow- and deep-rooted vascular plants (Fig. 5c), opposite its effect on brown moss and *Sphagnum* moss abundance (Fig. 5d). SEMs on plant functional types specially revealed the importance of calcium for *Sphagnum* mosses (richness and cover) and in turn their feedback effect on moss and overall plant community composition over the gradient (Fig. 5d, e, f). We further found that calcium significantly drove richness of small and large testate amoebae, as well as their community composition (Fig. 5g). SEMs especially showed that calcium highly impacted the abundance of large testate amoebae, which in turn negatively affect the community composition of small and overall testate amoebae (Fig. 5h).

SEM based on the pure relationships between plants and testate amoebae showed that *Sphagnum* mosses (abundance and richness) was good for predicting the abundance of large testate amoebae, and thus the community composition of testate amoebae through its indirect effect on plant community structure (Fig. 5i, j). Overall, SEMs indicate that higher calcium content leads to decreased abundance and richness of *Sphagnum* mosses and large testate amoebae, and that decreases in *Sphagnum* cover highly impact the function of testate amoeba community.

Discussion

Differences in functional traits of dominant plant functional types (PFTs) have a strong regulatory role in peatland processes (Lang *et al.* 2009; Ward *et al.* 2009; Turetsky *et al.* 2012). While it has been shown that PFTs drive ecosystem processes in peatlands (Kuiper *et al.* 2014), our study indicates a strong determining role of PFTs on soil microbial consumer community composition. As soil communities respond both to changes in PFT richness and abundance, changes in the PFT composition along the poor to extremely rich fen gradient may exert an important control on the community structure of testate amoebae, with cascading effects throughout the microbial food web, and ultimately to ecosystem processes like C and nutrient cycling.

Plant species richness, diversity and cover were highest in mesotrophic conditions (i.e. moderately/rich fen type), confirming earlier findings that intermediate values of water table depth, pH and calcium are the optimal niche conditions in terms of diversity for plant communities in fens (Hájková & Hájek 2004; Mälson, Backéus & Rydin 2008; Sekulová *et al.* 2013). As already shown in most of the central-European mires (Hájková & Hájek 2004; Hájek *et al.* 2006), we found pH, calcium and sulphates to be the main determinants of plant richness and community composition with negative effects on specifically plant types such as *Sphagnum* mosses. Our results show that *Sphagnum* mosses and shallow-rooted vascular plants are strongly affected in terms of richness and cover when the calcium content exceeds an apparent limit of around 50 mg. L^{-1}. This point then coincides when brown mosses start to replace *Sphagnum* mosses. Research on plants physiology showed that high concentrations of calcium in soils not only has toxic effects on vascular plants, but also on bryophytes (Bridgham *et al.* 1996). Although SEMs showed that calcium did not directly affect the vascular plants, it affected the mosses. Calcium is required for various structural roles in the cell wall, membranes and developmental cues, but in excessive concentration it directly reduce plant growth (White 2003). Indeed, Clymo and Hayward (1982) found reduced growth of *Sphagnum* mosses at high Ca^{2+} concentrations and relatively low pH (pH~6), showing a direct toxic effect. Calcium may also indirectly affect calci-tolerant *Sphagnum* species such as *S. warnstorfii* or *S. teres* by causing P deficiency through formation of Ca-P complexes lowering plant availability (Bridgham *et al.* 1996; Hájková & Hájek 2004). However, such calci-tolerant species were only recorded in few plots, suggesting the Ca^{2+} effect on *Sphagnum* was rather direct in our study. Sulphates also emerged as a significant co-variable explaining *Sphagnum* cover. This agrees with previous studies in mires where high sulphate content was found to impact the photosynthetic capacity of *Sphagnum* by damaging PSII system (Granath, Wiedermann & Strengbom 2009), resulting in a reduction in *Sphagnum* productivity and eventually the *Sphagnum* cover.

Testate amoebae showed different patterns of richness, diversity, and relative abundance between poor- to extremely rich fen conditions. These patterns were explained by changes in environmental conditions, most evidently at the start of the gradient. In bogs and fens, water table depth, pH and minerals were identified as key control on testate amoeba communities (Opravilova & Hájek 2006; Lamentowicz *et al.* 2011), although their role has not been explained sufficiently on the physiological level. Striking is the divergent response of small and large testate amoeba species along the gradient. While increasing minerotrophic conditions may lead to a loss of locomotor activity of large naked amoebae (Gollnick, Meyer & Stockem 1991)—which could explain why large taxa decreased along the gradient—our findings indicate that changes in PFT cover could also drive their decrease. Although plant composition reflects indirect effects of calcium and sulphates on its community structure, moving-window analyses showed the dependence of testate amoebae to abiotic conditions to decrease over the gradient, while their dependence to plants remained high and stable. These findings are in agreement with our hypothesis, and the tight coupling between plant and testate amoeba communities suggests these communities synchronously change over the gradient. This may relate to high frequency and specificity of plant-testate amoeba interactions, especially with bryophytes. After removing the effect of calcium on plant-testate amoeba linkages, we show that moss types highly drive niche-size structure of testate amoebae. Recent studies have emphasized the importance of mosses for microbial communities in peatlands, including testate amoebae (Lamentowicz *et al.* 2010; 2011). Here, we found that a decrease in *Sphagnum* cover greatly impacted the relative abundance of large testate amoebae, which tend to disappear in rich to extremely rich fens.

On the contrary, small taxa rather followed brown moss cover and increased along the poor-rich gradient. This shows that testate amoebae adapt their size-distribution to that of bryophyte species composition and indirectly to calcium concentrations. Morphological and niche differences between brown and *Sphagnum* species can easily explain such patterns but alternative hypotheses should also be considered. Our results raise the question if there could be an autogenous influence of mosses on specific testate amoeba development. It was recently shown that bryophytes chemically interact with their surrounding environment by releasing chemical compounds such as polyphenols (Chiapusio *et al.* 2013; Bay *et al.* 2013). Recent research on the organochemical compounds underlined the ability of *Sphagnum* and brown mosses to chemically interact with other plants (Soudzilovskaia *et al.* 2011) or their associated microbial communities (Bay *et al.* 2013), including testate amoebae (Chiapusio *et al.* 2013). These allelochemical effects can even exceed those of environmental factors (Jassey *et al.* 2011). As bryophytes can produce and release species-specific polyphenols in their surrounding (Abbott *et al.* 2013), we hypothesized that specific-allelochemical compounds have a strong influence on belowground communities, which may explain the relationships between the size-structure of the testate amoeba communities and moss types in our study. Nevertheless, we cannot rule out the influence of environmental and morphological factors, yet these controls may be additive. Furthermore, it was shown that bryophyte community composition has knock-on effects on vascular plant populations via generative reproduction (Soudzilovskaia *et al.* 2011), suggesting that complex allelochemicals interactions occurred between mosses, vascular plants and testate amoebae along the poor-rich gradient.

We found that interactions between testate amoebae, mosses and vascular plants differed along the gradient. Usually, testate amoebae are linked better to mosses than vascular plants (Lamentowicz *et al.* 2010; 2011). Mosses, when surrounded with a water film, create suitable conditions for many microbial communities, including testate amoebae (Gilbert *et al.* 1998), while various species of vascular plants such as sedges and rushes have long, flat and usually dry leaves, making them relatively inhospitable for microorganisms that occur mostly in the detrital layer of the soil or in the open water of wet hollows. Yet, moving window analysis showed that shifts in shallow-rooted plants were strongly related to changes in testate amoeba assemblages up to the middle of the gradient. This suggests that the influence of root-exudation on soil microfauna assemblages decreases with the concomitant reduction of shallow-rooted plant species. SEMs did not reveal significant causal effect of shallow-rooted plants on testate amoebae. We envisage that increasing small testate amoeba species, which consume fauna of lower trophic levels such as bacteria and fungi, induced trophic cascades on decomposition and nutrient mineralization that could indirectly impact growth of shallow-rooted vascular plants (Bardgett *et al.* 2013).

Owing to the low density and diversity of meso- and macrofauna in peatlands, most biogeochemical cycles in mires rely on the moss associated microbial food web (Lamentowicz *et al.* 2013). Mosses play an important role in nutrient and carbon cycling as they capture and accumulate detritus (Turetsky *et al.* 2012), fix C from atmospheric pools (Robroek *et al.* 2009), and compete with vascular plants for N availability (Berendse *et al.* 2001). However, differences in size-distribution of dominant microbial consumers (testate amoebae) are likely to modify such processes (Jassey *et al.* 2013). Consumers show a consistent size structure characterized by increases in body size with trophic levels (i.e. predators become larger along food chains) (Dossena *et al.* 2012). Decreases in population and/or community averaged size structure (e.g. body size) indicate a loss of high trophic levels within food webs (Ohlberger 2013). Such variations in community size-structure were found to significantly influence fluxes of ecosystem respiration and gross primary production in ecosystems (Yvon-Durocher & Allen 2012). Therefore, our results suggest that niche-size exclusion of testate amoeba communities along the gradient may have knock-on effects on biogeochemical processes in the different fen types, which in turn have the potential to regulate the availability of nutrients for plants, and therefore conceivably affect their growth. Impact of plant functional types on size-structure of testate amoebae, and thus microbial food web, could also be a mechanism explaining why divergent plant communities affects gas fluxes in peatlands (Ward *et al.* 2009; 2013; Kuiper *et al.* 2014).

Conclusions

The relationships between plant functional types and testate amoeba communities evidenced in this study provide additional support for the importance of linkages between plant and microbial communities to peatland functioning. Our results show that in ecosystem where bryophytes are considered as ‘ecosystem-engineers’ (Van Breemen 1995), the impacts of vascular plants on key components of ecosystem function such as testate amoeba community structure differed following their functional traits such as root-length (Kuiper *et al.* 2014). Determining mechanisms explaining such findings was not the aim of this study, even if the quantity and quality of root exudates inputs and moss secondary metabolites remains potential factors driving succession of microbial organisms in fens. Our study shows that calcium concentration and the peatland bryophyte community composition mainly drive niche-size structure of testate amoebae across a poor to rich fen gradient. These changes in the dominant microbial consumer community composition could have key impact on biogeochemical processes such as carbon cycling. Assessing size-structure and the factors that control it (e.g. allelochemical interactions), therefore will further aid our understanding on the links between plant functional types, soil organisms and biogeochemical cycles in fens.

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Figures captions

Figure 1: (**a**) Map of Poland showing the location of the eight fens from the Wielkopolska region, western Poland (dark grey area) and the magnitude (size scale) of main abiotic factors across each fens: DWT, pH, Calcium, nitrogen (NH4 + NO3), phosphates, and sulphates. Dot sizes on individual maps are directly proportional to the mean values of specific factor in each fens but are not comparable among maps. (**b**) Dendrogram resulting from cluster analysis (Ward's method) based on physico-chemical component of water. Particular fen types are marked: PR – poor fen, MRF – moderately rich fen, RF – rich fen, ERF – extremely rich fen.

Figure 2: Redundancy analysis plots of moss (**a**), vascular plant (**b**) and testate amoeba (**c**) Hellinger-transformed species data constrained by abiotic water-chemical variables from the Wielkopolska region fens, Western Poland. Abiotic variables retained after model selection are represented by vectors: DWT : depth to water table ; Ca = calcium. PR – poor fen, MRF – moderately rich fen, RF – rich fen, ERF – extremely rich fen.

Figure 3: Diversity, richness, and cover/relative abundance (mean ± SEM) of moss functional types (**a, b, c**), vascular plant types (**d, e, f**), and testate amoeba types (**g, h, i**) over different fen types. Different letters indicate significant differences over fen types ($P < 0.05$); small letters for different vascular plant and moss functional types, and testate amoeba size species, capital letters overall plant, moss and testate amoebae. PR – poor fen, MRF – moderately rich fen, RF – rich fen, ERF – extremely rich fen. Abbreviations for species are given in Supplementary Table 2 and 3.

Figure 4: (**a**) Moving-window redundancy analysis of the causal relationships between moss, vascular plant and testate amoeba Hellinger-transformed data sets and abiotic factors identified in RDAs along the poor- to extremely rich fen gradient. (**b**) Moving-window redundancy analysis of the causal relationships between testate amoeba, total plant, vascular plant and moss Hellinger-transformed data sets, respectively. (**c, d**) Moving-window redundancy analysis of the causal relationships between large and small testate amoeba, and plant functional type (*Sphagnum* vs. brown mosses and shallow- and deep-rooted plants) Hellinger-transformed data sets. The horizontal axis indicates the position of

the moving-window over which the explained variation was calculated along the gradient (poor- to extremely rich fens): 1: sites 1-70, 2: sites: 2-71, 3: sites 3-72… 70: sites 62-132. Significant relationships are pointed out by solid symbols ($P < 0.05$). Abbreviations are: Amo, testate amoebae; Env, abiotic factors; Veg, total plants; Mos, mosses; Vas, vascular plants; Sph, Sphagnum; Brown, brown mosses; Shal, shallow-rooted plants; Deep, deep-rooted plants; Large, large testate amoebae; Small, small testate amoebae. All communities were previously re-ordered following the calcium content to reflect the poor- to extremely rich fen gradient.

Figure 5: Minimal adequate models for the effects of calcium on plant community composition and testate amoeba size structure, as well as the effect of plant functional types on testate amoeba size structure (see Supplementary Figure 3 for the maximal model). Solid arrows show significant relationships (pathways) between variables, dotted arrows indicate a nonsignificant relationship, and numbers next to arrows show standardized parameter estimates (i.e., standardized regression weights). Circles (e1–e5) indicate error terms, and double-headed arrows indicate significant correlations between the error terms. Squared multiple correlations (R^2) for the predicted/dependent factor is given on the box of the dependent variable. Fit indices are given in Supplementary Table 6. Abbreviations are: Div, Shannon's diversity; AB, abundance; Rich, species richness; compo, community composition; Plant, total plant; Veg, total plants; Mos, mosses; Vas, vascular plants; Sha: shallow-rooted plants; Sph: *Sphagnum* mosses; Amo: testate amoebae; La: large; Sma: small.

Table 1: Location data of fens from the Wielkopolska region, western Poland.

Site	**Longitude**	**Latitude**	**Altitude**	**Size (ha)**	**Trophic state**	**Type of microhabitat**	**Number of samples**
Kuźnik Bagienny (KB)	53°12’51’’	16°43’52”	76 m	0.5	Poor fen	*Sphagnum* lawns; *Sphagnum* hummocks; floating mat margins	9
Kuźnik Olsowy (KO)	53°12’46’’	16°43’39’’	83 m	0.4	Moderately rich fen	Floating mat; *Sphagnum* and brown moss lawns;	22
Makąty (MAK)	52°39’23”	15°52’20”	42 m	4.7	Moderately rich fen	Floating mat; *Sphagnum* and brown moss lawns;	7
Rurzyca (RUR)	53°17’12”	16°43’18”	85 m	3.5	Rich fen	Flooded hollows, *Sphagnum* and brown moss lawns, and hummocks	23
Wagowo (WAG)	52°25’11”	17°21’53”	125 m	3	Rich fen	Floating-mat, flooded hollows, *Sphagnum* and brown moss lawns, and hummocks	19
Wierzchołek (WEK)	53°24’56”	17°14’9”	114 m	1.2	Rich fen	Flooded hollows, brown moss lawns, and hummocks	14
Czarne (CZAR)	52°28”28”	17°53’22”	100 m	27.1	Extremely rich fen	Floating-mat; flooded hollows, *Sphagnum* and brown moss lawns, and hummocks	18
Kazanie (KAZ)	52°27’30’’	17°17’53”	106 m	2.4	Extremely rich fen	Floating-mat; flooded hollows, *Sphagnum* and brown moss lawns, and hummocks	20
Sum							**132**

Table 2: Components of hypotheses represented by structural equation models

Model	**Hypothetical pathways**
a, b	Calcium content directly influences vegetation composition (Hájek *et al.* 2006; Sekulová *et al.* 2013).
a	Calcium content indirectly influences vegetation composition through its effect on plant diversity, which in turn drives vascular plant and moss diversity (Hájek *et al.* 2006; Sekulová *et al.* 2013).
b	Calcium content indirectly influences vegetation composition through its effect on vascular plant and moss community compositions (Hájek *et al.* 2006; Sekulová *et al.* 2013).
c	Calcium content directly influences the cover of plant functional types of vascular plants, which in turn drives vascular plant community composition (Hájek *et al.* 2006; Sekulová *et al.* 2013).
d	Calcium content directly influences the cover of plant functional types of mosses, which in turn drives moss community composition (Hájek *et al.* 2006; Sekulová *et al.* 2013).
e	Calcium content influences the richness of plant functional types (moss and vascular plant), which in turn drives vegetation community composition.
f	Calcium content influences the cover of plant functional types (moss and vascular plant), which in turn drives vegetation community composition.
g, h	Calcium content directly influences testate amoeba community composition (Lamentowicz *et al.* 2011).
g	Calcium content influences the richness of small and large testate amoebae, which in turn drive testate amoeba community composition.
h	Calcium content influences the abundance of small and large testate amoebae, which in turn drive testate amoeba community composition.
i	The richness of plant functional types drives testate amoebae richness and large testate amoeba abundance, which in turn determine testate amoeba community composition along the gradient.
j	The abundance of plant functional types drives vegetation community composition, which in turn determines testate amoeba community composition along the gradient.
j	The abundance of plant functional types influences the abundance of large testate amoebae, which in turn determines testate amoeba community composition along the gradient.

Table 3: Rank correlation between vascular plant (deep- and non-deep-rooted plants) and moss (*Sphagnum* and brown mosses) and testate amoeba (small and large) species richness and diversity. Below diagonal (bottom-left-half of the matrix): Spearman’s correlation, above diagonal: Holm-corrected *P*-value. Significant correlations appear in bold.

	Brown mosses	*Sphagnum* mosses	Shallow-rooted plants	Deep-rooted plants	Large-sized testate amoebae	Small-sized testate amoebae
Species richness N0						
Brown mosses	1	0.003	0.009	0.183	0.016	< 0.001
Sphagnum mosses	**-0.254**	1	0.912	0.212	0.266	< 0.001
Shallow-rooted plants	**0.226**	0.010	1	0.193	0.887	0.963
Deep-rooted plants	0.117	0.109	0.114	1	0.995	0.249
Large-sized testate amoebae	**0.209**	-0.097	0.013	0.001	1	0.004
Small-sized testate amoebae	**0.424**	**-0.402**	-0.004	0.101	**0.247**	1
Shannon's diversity N1						
Brown mosses	1	0.821	0.002	0.318	0.008	< 0.001
Sphagnum mosses	-0.020	1	0.831	0.195	0.851	0.208
Shallow-rooted plants	**0.262**	0.019	1	0.137	0.505	0.659
Deep-rooted plants	0.088	0.113	0.130	1	0.447	0.916
Large-sized testate amoebae	**0.229**	0.016	0.058	-0.067	1	0.001
Small-sized testate amoebae	**0.417**	-0.110	0.039	0.009	**0.276**	1

Figure 1

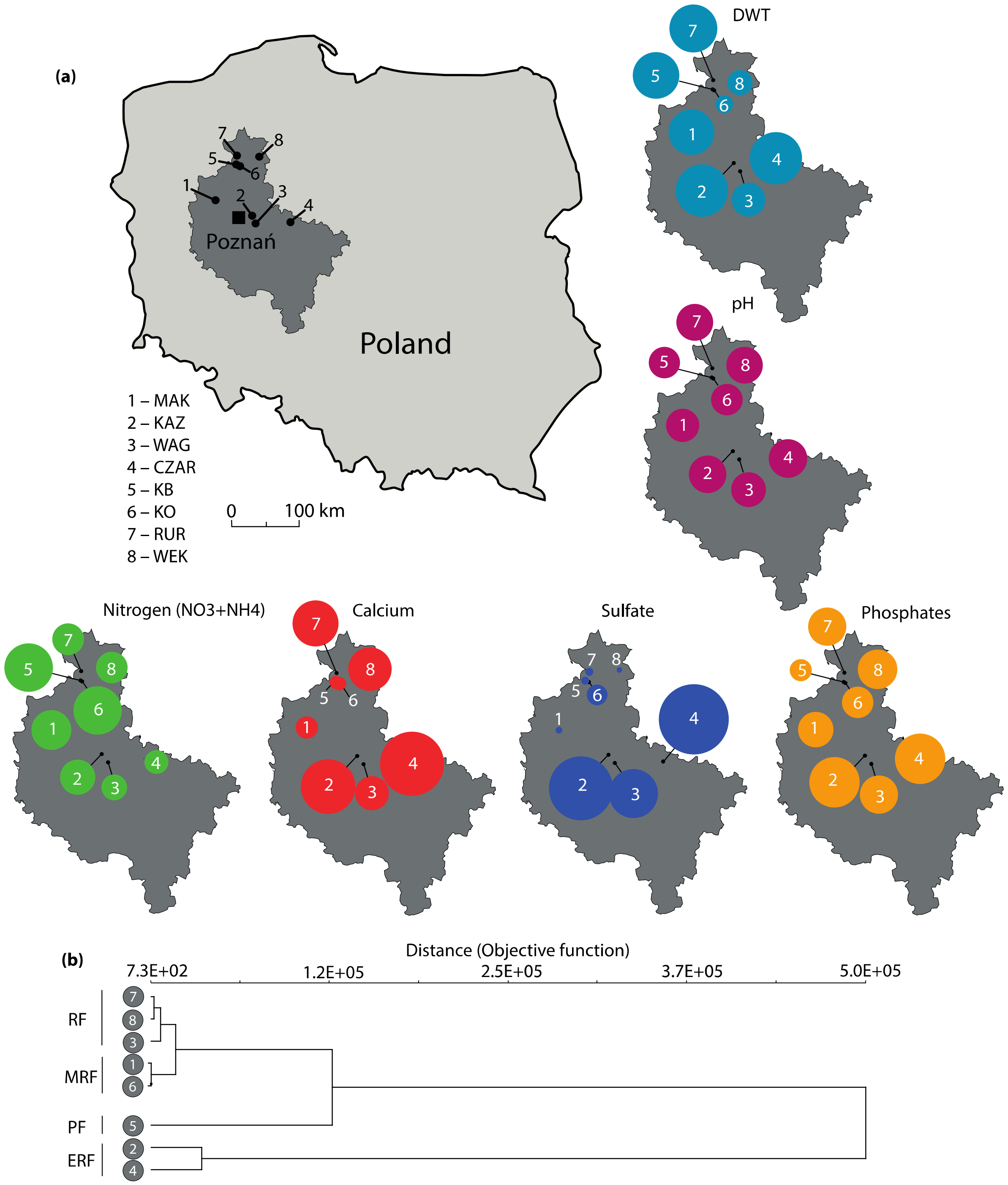

(a)
Poland
Poznań
1 – MAK
2 – KAZ
3 – WAG
4 – CZAR
5 – KB
6 – KO
7 – RUR
8 – WEK
0
100 km
DWT
pH
Nitrogen (NO3+NH4)
Calcium
Sulfate
Phosphates
(b)
Distance (Objective function)
7.3E+02
1.2E+05
2.5E+05
3.7E+05
5.0E+05
RF
MRF
PF
ERF
7
8
3
1
6
5
2
4

Figure 2

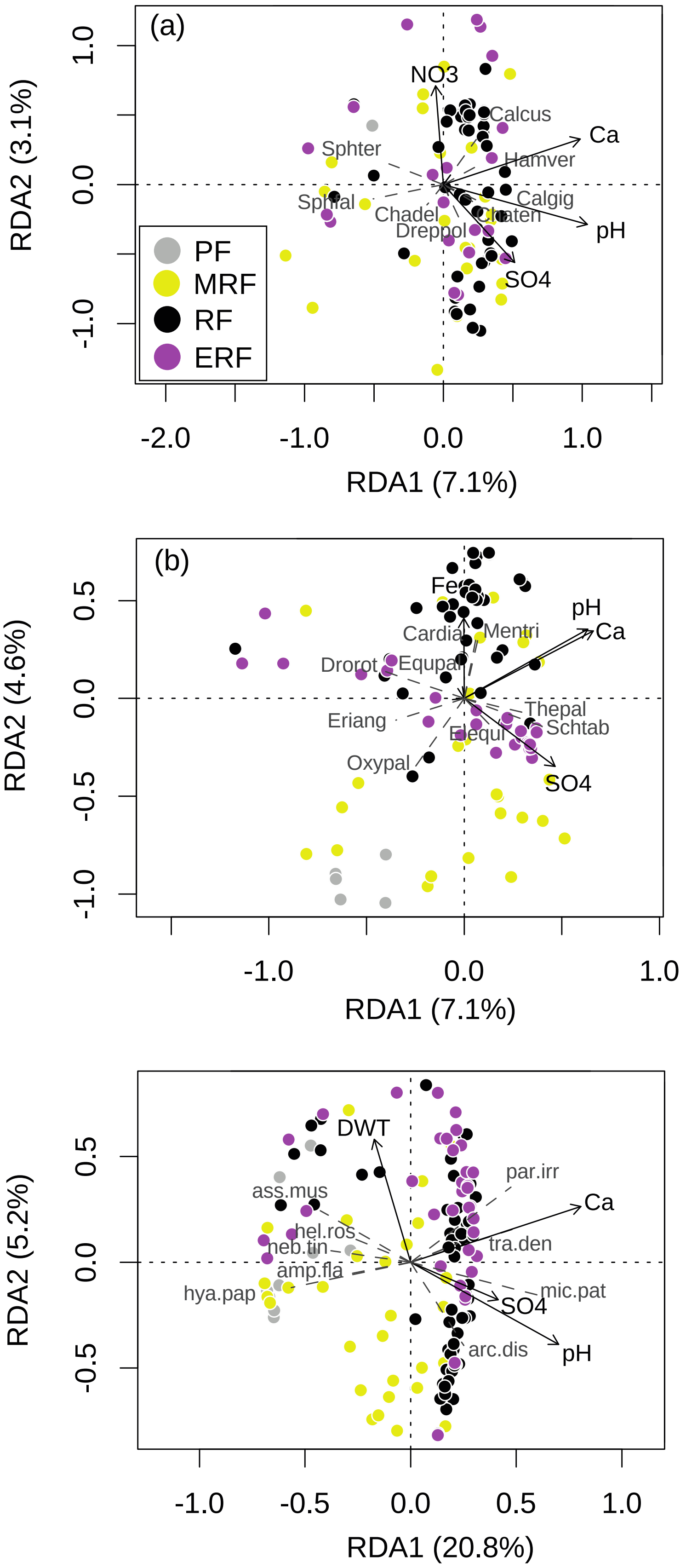
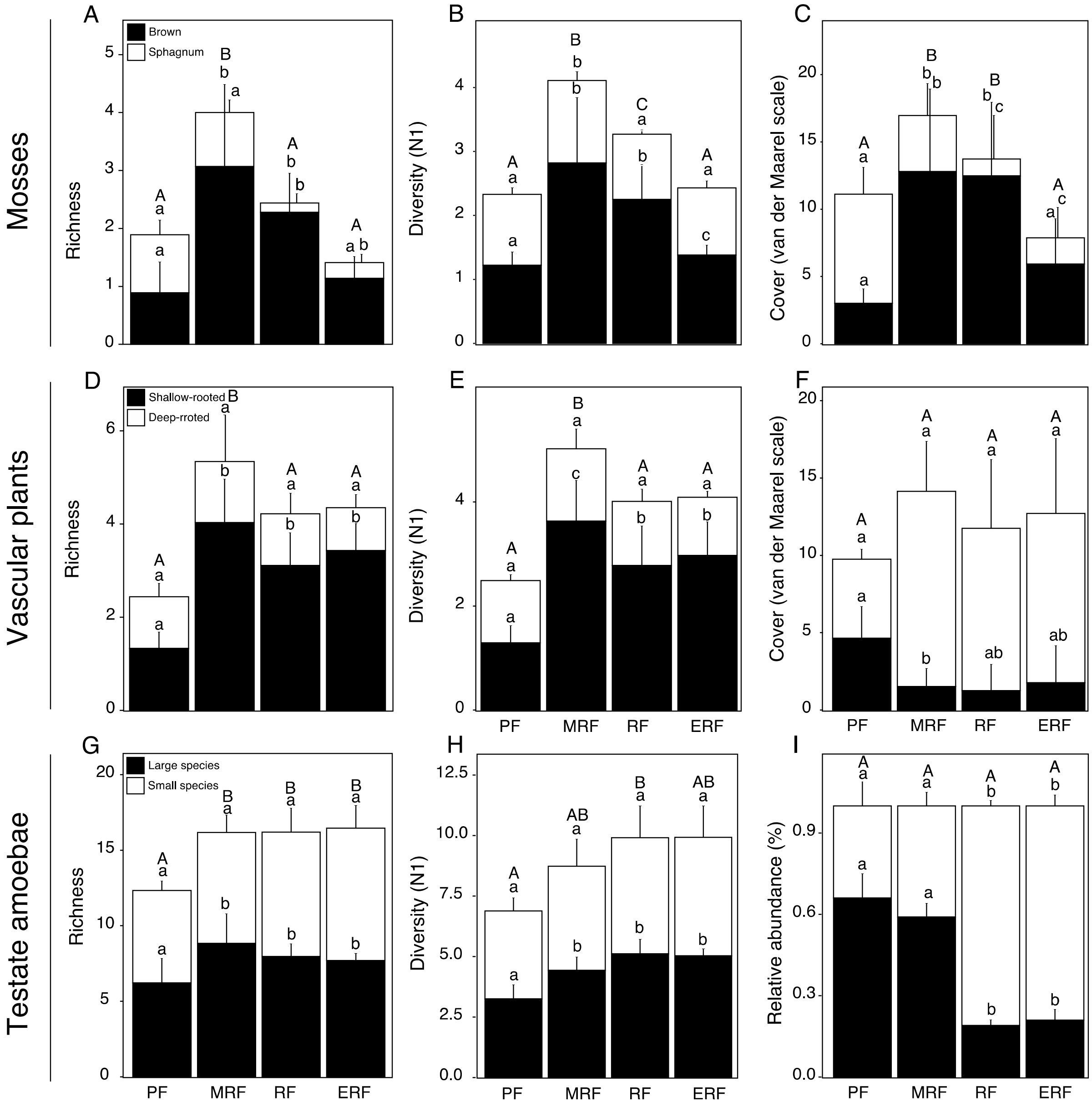

(a)
RDA2 (3.1%)
RDA1 (7.1%)
1.0
0.0
-1.0
-2.0
-1.0
0.0
1.0
NO3
Calcus
Ca
Sphter
Hamver
Calgig
Sphfal
Chadel
Chaten
pH
Dreppol
SO4
PF
MRF
RF
ERF
(b)
RDA2 (4.6%)
RDA1 (7.1%)
0.5
0.0
-0.5
-1.0
-1.0
0.0
1.0
Fe
pH
Cardia
Mentri
Ca
Drorot
Equpal
Thepal
Eriang
Schtab
Elequi
Oxypal
SO4
RDA2 (5.2%)
RDA1 (20.8%)
0.5
0.0
-0.5
-1.0
-0.5
0.0
0.5
1.0
DWT
par.irr
ass.mus
Ca
hel.ros
tra.den
neb.tin
amp.fla
hya.pap
mic.pat
SO4
arc.dis
pH

Figure 3



Mosses
Vascular plants
Testate amoebae
A
B
C
D
E
F
G
H
I
Brown
Sphagnum
Shallow-rooted
Deep-rroted
Large species
Small species
Richness
Diversity (N1)
Cover (van der Maarel scale)
Relative abundance (%)
PF
MRF
RF
ERF
0
1
2
3
4
5
6
10
15
20
0.0
2.5
5.0
7.5
10.0
12.5
0.3
0.6
0.9

Figure 4

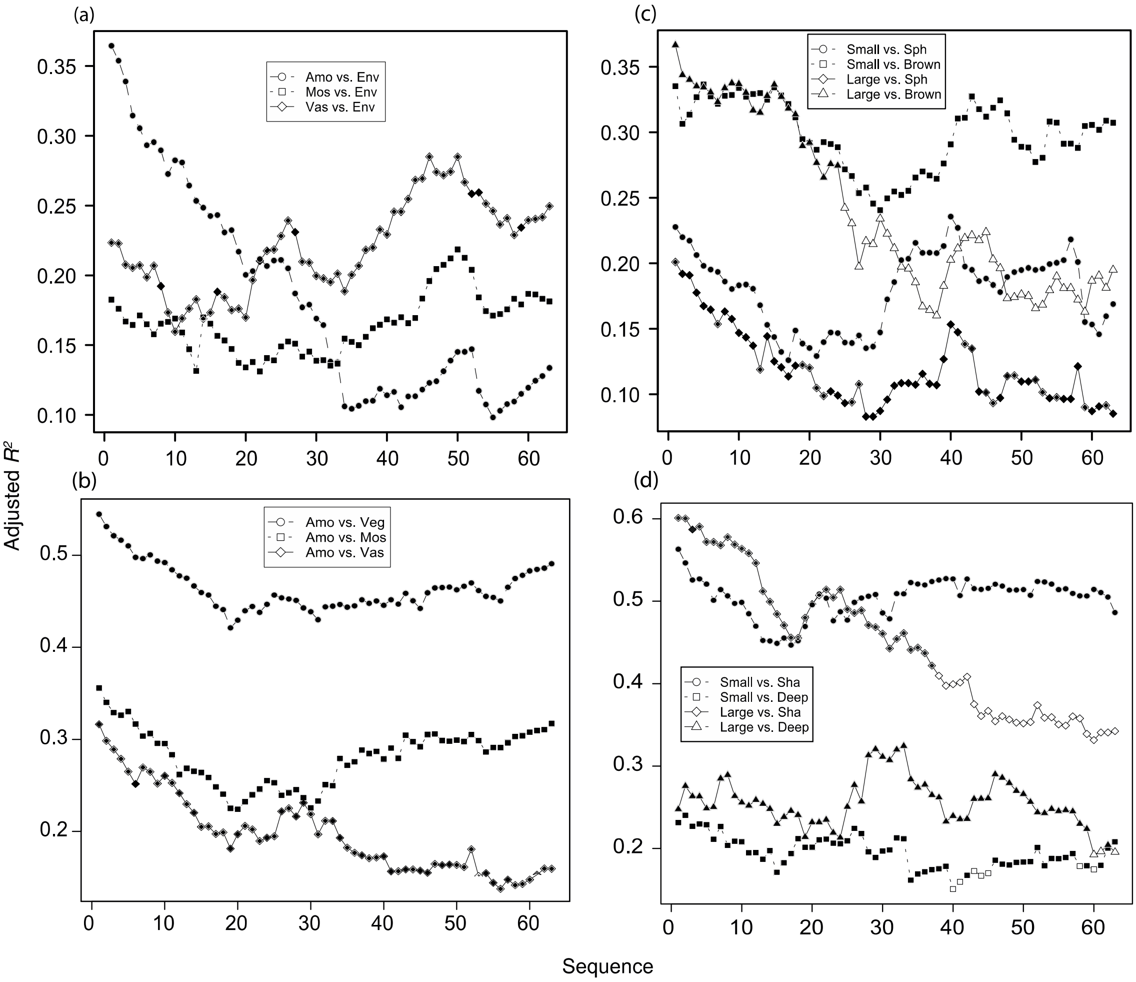
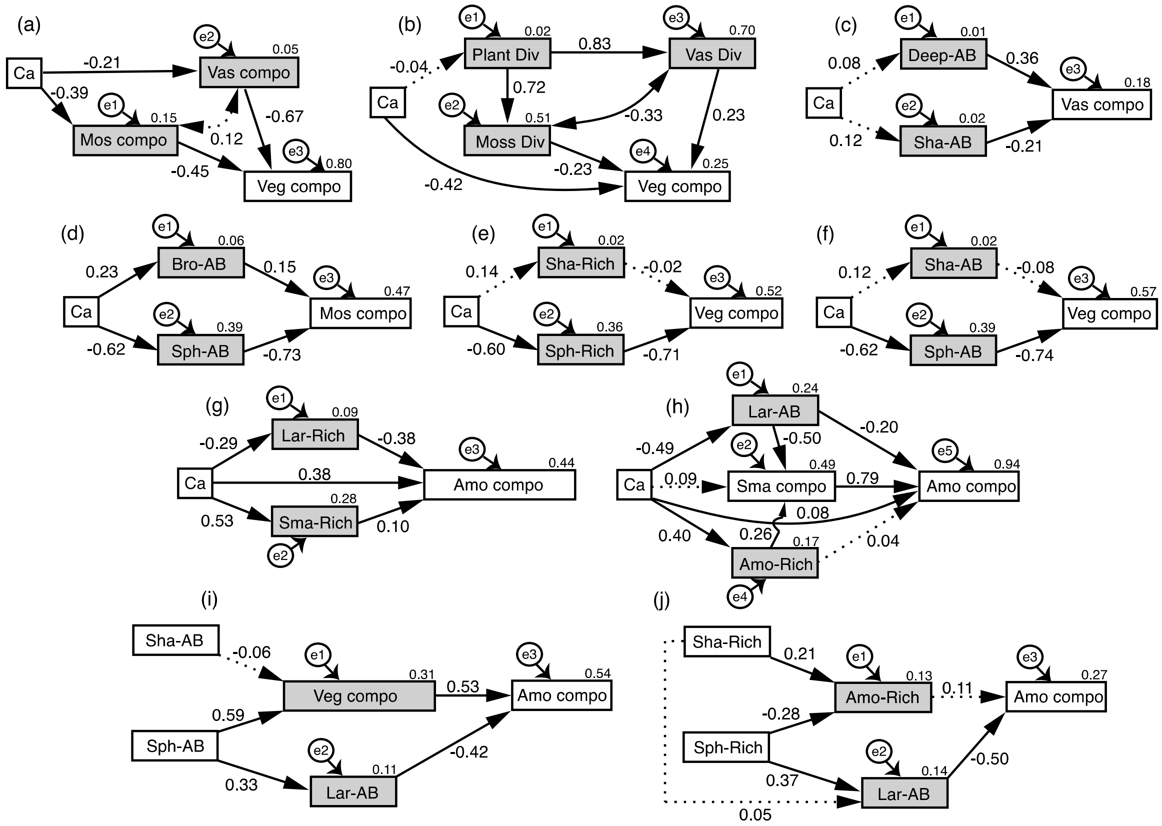

(a)
Amo vs. Env
Mos vs. Env
Vas vs. Env
0.35
0.30
0.25
0.20
0.15
0.10
0
10
20
30
40
50
60
(c)
Small vs. Sph
Small vs. Brown
Large vs. Sph
Large vs. Brown
0.35
0.30
0.25
0.20
0.15
0.10
0
10
20
30
40
50
60
Adjusted R^2
(b)
Amo vs. Veg
Amo vs. Mos
Amo vs. Vas
0.5
0.4
0.3
0.2
0
10
20
30
40
50
60
(d)
Small vs. Sha
Small vs. Deep
Large vs. Sha
Large vs. Deep
0.6
0.5
0.4
0.3
0.2
0
10
20
30
40
50
60
Sequence

Figure 5


(a)
Ca
-0.21
Vas compo
e2
0.05
-0.39
e1
0.15
Mos compo
0.12
-0.67
e3
0.80
-0.45
Veg compo
(b)
e1
0.02
Plant Div
0.83
e3
0.70
Vas Div
-0.04
Ca
0.72
e2
0.51
Moss Div
-0.33
0.23
e4
0.25
-0.23
-0.42
Veg compo
(c)
e1
0.01
Deep-AB
0.08
0.36
e3
0.18
Ca
Vas compo
e2
0.02
0.12
Sha-AB
-0.21
(d)
e1
0.06
Bro-AB
0.23
0.15
e3
0.47
Ca
Mos compo
e2
0.39
-0.62
Sph-AB
-0.73
(e)
e1
0.02
Sha-Rich
0.14
-0.02
e3
0.52
Ca
Veg compo
e2
0.36
-0.60
Sph-Rich
-0.71
(f)
e1
0.02
Sha-AB
0.12
-0.08
e3
0.57
Ca
Veg compo
e2
0.39
-0.62
Sph-AB
-0.74
(g)
e1
0.09
Lar-Rich
-0.29
-0.38
e3
0.44
Ca
0.38
Amo compo
0.28
0.53
Sma-Rich
0.10
e2
(h)
e1
0.24
Lar-AB
-0.49
-0.50
-0.20
e2
0.49
e5
0.94
Ca
0.09
Sma compo
0.79
Amo compo
0.08
0.40
0.26
0.17
0.04
Amo-Rich
e4
(i)
Sha-AB
-0.06
e1
0.31
e3
0.54
Veg compo
0.53
Amo compo
0.59
Sph-AB
e2
0.11
-0.42
0.33
Lar-AB
(j)
Sha-Rich
0.21
e1
0.13
e3
0.27
Amo-Rich
0.11
Amo compo
-0.28
Sph-Rich
e2
0.14
-0.50
0.37
Lar-AB
0.05