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**Creevy, AL, Fisher, J, Puppe, D and Wilkinson, DM (2016) Protist diversity on a nature reserve in NW England – with particular reference to their role in soil biogenic silicon pools. Pedobiologia. ISSN 0031-4056**

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## Accepted Manuscript

Title: Protist diversity on a nature reserve in NW England – with particular reference to their role in soil biogenic silicon pools

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PII: S0031-4056(16)30003-8  
DOI: <http://dx.doi.org/doi:10.1016/j.pedobi.2016.02.001>  
Reference: PEDOBI 50453

To appear in:

Received date: 3-12-2015  
Revised date: 2-2-2016  
Accepted date: 3-2-2016

Please cite this article as: Creevy, Angela L., Fisher, Jane, Puppe, Daniel, Wilkinson, David M., Protist diversity on a nature reserve in NW England *minus* with particular reference to their role in soil biogenic silicon pools. *Pedobiologia - International Journal of Soil Biology* <http://dx.doi.org/10.1016/j.pedobi.2016.02.001>

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## Protist diversity on a nature reserve in NW England – with particular reference to their role in soil biogenic silicon pools

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### Highlights

- Considering only a few groups of microorganisms greatly increases the known diversity of a nature reserve.
- This microbial diversity is important in many ecological processes.
- Diatoms and some testate amoebae create hotspots of Si in the soil.
- Testate amoebae are particularly important in mor humus soils.
- Quantifying microbial population sizes provides data that can be used to start to estimate their functional role in the soil.

### ABSTRACT

Soil protists play fundamental roles in many earth system processes, yet we are only beginning to understand the true diversity of the organisms involved. In this study we used conventional (microscopy-based) methods to characterise the diversity and estimate protist population sizes in soils from a variety of distinct habitats within Mere Sands Wood nature reserve in NW England. We produced population size data for over ninety soil protists belonging to two major eukaryotic functional groups: testate amoebae (TA) and diatoms, adding substantial ‘cryptic diversity’ to the nature reserves recorded biota. From these population size data we estimated relative contributions of TA and diatoms to soil biogenic silicon (BSi) pools and found significant correlations between taxon richness and the TA and diatom Si pool. This could indicate that protist functional diversity can influence terrestrial BSi pools, especially in early successional plant communities where TA and diatoms can potentially increase Si

mineralisation and/or create Si ‘hot spots’ and hence, the biological availability of this element for subsequent plant uptake. TA were particularly abundant in mor humus type soils further supporting the idea that they could be important players in nutrient cycling in such soils. Overall, we demonstrate this is a useful approach in order to start to attempt to estimate the role of protists in the Si cycle and other ecological processes.

**KEYWORDS:** protist, population size, diversity, testate amoebae, diatoms, biogenic silicon.

## 1. Introduction.

Over the last 40 years there has been a profound shift in the way in which many scientists view the role of life in the context of the whole Earth – from life being viewed as just inhabiting the planet, to it playing major roles in the workings of the Earth System. This systems view of the Earth mainly developed during the 1980’s and 90’s and was influenced by several sources; including the extra-planetary perspective that came from NASA’s Apollo Missions and the Gaia hypothesis developed by James Lovelock and Lynn Margulis in the early 1970’s (Hamilton and Grinevald, 2015). From the start what was to become Earth Systems science – especially as envisaged by the Gaia hypothesis put a strong emphasis on the role of microorganisms in the Earth system (Margulis and Lovelock, 1974). Lovelock (1993) also argued that soils made an interesting model for the planet as a whole, because life plays a major role in constructing soils rather than merely adapting to abiotic soil conditions. Indeed the development of soils and the associated vegetation cover may have played significant roles in Earth history through the climatic effects of the weathering of soil minerals on the global climate (Schwartzman and Volk, 1989; Lenton et al., 2012). However, while clearly of crucial importance, the ecology of soils in general, and soil microbes in particular, was until recently somewhat understudied. As one of us wrote a few years ago: ‘The soil is largely ‘out of sight’ to an ecologist without a spade and, for much of the 20<sup>th</sup> century, this has meant that it was also ‘out of mind’ to most ecologists. At best, it tended to be treated as a black box, with the behaviour of its inhabitants lumped together under simple labels such as decomposers or nitrogen fixers’ (Wilkinson, 2008, p596). Even within microbial ecology soil protists have been far less studied than bacteria or fungi – although there are some signs that this is starting to change (Wilkinson et al., 2012). In some recent studies both traditional approaches (Finlay and Fenchel, 2001; Domonell et al., 2013; Geisen et al., 2014; Andriuzzi et al., 2016) and modern (Geisen et al., 2015; Lara et al., 2015) sequence-based analyses have been applied to study natural protist communities in soils.

From before the full development of the current systems approach to global ecology it was known that life was sustained by the cycling of energy and chemical elements (e.g. Hutchinson, 1970), however a systems approach can be seen as putting particular emphasis on the merging of organismal and ecological physiology (Wilkinson, 2003; 2006). To understand such processes we need to be able to quantify microbial diversity, population sizes and biomass. Given that, as Sean Nee (2004, p 804) emphasised, ‘the contribution of visible life to

biodiversity is very small indeed' this is no small task – much of the life we need to quantify to understand the world is microscopic. This vast expanse of microscopic biological diversity forms part of what has been called 'cryptic diversity' and is not included in most conservation surveys (Esteban and Finlay, 2010). Indeed the microbial aspect of this cryptic diversity is seldom assessed in biodiversity surveys – it's unusual to have good data on even the small metazoan fauna and fungi (e.g. Corbet, 2011). One substantial and understudied aspect of this cryptic diversity in soils is composed of protists (Geisen et al., 2015). A number of papers from the 1950's to the 1970's provided some limited estimates of Testate amoebae production in soils – especially the work by Bonnet and Thomas (1955); Lousier and Parkinson (1984) and Schönborn (1965, 1978, 1983), this work is extensively summarised and evaluated by Wilkinson and Mitchell (2010). As Heger et al. (2014) have argued, there needs to be a resurgence in field research on microbial eukaryotes because this area of study is currently limited by the shortage of data on natural protist communities – especially heterotrophic protists.

In this study we constrain the problem to manageable proportions by considering just two functional groups of protists – testate amoebae (TA) and diatoms, and use the silicon (Si) cycle as an example of the potential importance of soil protists. Various pro- and eukaryotes are evolutionarily adapted to synthesize siliceous structures (biosilicification). These organisms use monomeric silicic acid ( $H_4SiO_4$ ) for synthesis of biogenic silica, i.e. hydrated amorphous silica ( $SiO_2 \cdot nH_2O$ ) (e.g. Ehrlich et al., 2010). In terrestrial ecosystems the accumulation of biogenic silica of microbial (bacteria, fungi), phytogetic (plants), protophytic (diatoms), protozoic (TA) and zoogenic (sponges) origin results in formation of corresponding biogenic Si (BSi) pools (Puppe et al., 2015; Sommer et al., 2006). TA form a polyphyletic group traditionally placed in the phylum Rhizopoda (Margulis and Chapman, 2009) but now split between two Eukaryote supergroups, the Amoebozoa (for the Arcellinida) and SAR (for the Euglyphida and other taxa with filose pseudopodia) (Adl et al, 2012). The members of the Euglyphida make self-secreted siliceous plates (idiosomes) (Ogden and Hedley, 1980). The diatoms also form part of the SAR eukaryotic supergroup forming the Diatomea (classically the Bacillariophyta) in the classification of Adl et al. (2012). These are predominately marine or freshwater photosynthetic microbes with tests (frustules) formed from amorphous silica and so require dissolved Si for growth (Margulis and Chapman, 2009). Although primarily aquatic, in some situations they may be important for retaining BSi in soils and peats (Kokfelt et al, 2009; Alfredsson et al., 2015). Accumulation and recycling of BSi in terrestrial ecosystems influence fluxes of dissolved Si from the continents to the oceans, thus act as a filter in the global Si cycle (Dürr et al., 2011; Struyf and Conley, 2012). Although this biogenic control mechanism has been generally recognized for decades (e.g. Bartoli, 1983; Meunier et al., 1999; Struyf and Conley, 2012) quantitative information on particular terrestrial BSi pools are surprisingly rare (Sommer et al., 2006). We hypothesise that diversity of TA and diatoms differs within spatially heterogenic terrestrial ecosystems (i.e. various habitats within a single nature reserve) and this, in turn, leads to corresponding patterns in the protophytic and protozoic Si pools. The aims of our work were: 1) to quantify soil protist diversity and population sizes in various habitats and 2) to investigate relationships between soil protist diversity and corresponding protophytic and protozoic Si pools.

## 2. Methods

### 2.1 *Study site and sampling procedure*

Mere Sands Wood (MSW) Nature Reserve (53°63'55" N, 2°83'71" W) is an artificial, post sand-mined catchment situated in the county of Lancashire in North West England. The soils of MSW have an interesting geological history and provide the best sections available for understanding the deposition of the Shirdley Hill Sands of Lancashire laid down during the late glacial and early post glacial periods (Chiverell et al., 2004). Sand extractors recognised the commercial value in the glass making industry and between 1974 and 1982 the site was quarried. Extracted areas were then landscaped into shallow-edged lakes. This spatially heterogeneous site is now managed as a nature reserve and is characterised by a series of artificially created lakes surrounded by deciduous and coniferous woodland with smaller patches of grassland, heathland and scrub. This is the first study investigating soil protists on the nature reserve. Field sampling was undertaken over two consecutive days in September 2011 and directed towards capturing the range of environmental variability within terrestrial habitats on the 42 hectare reserve. Four broad habitat types were identified: (DW) early successional deciduous woodland comprised open Silver birch (*Betula pendula*) canopy with a ground cover of grasses (Poaceae spp.) and bryophytes on a sandy substrate, (CW) artificially planted mature Scots pine (*Pinus sylvestris*) woodland with ground cover comprised of male fern (*Dryopteris* spp.) and bramble (*Rubus fruticosus* agg.), (G) Mesotrophic grassland mainly comprised of grasses (Poaceae spp.) and Rushes (*Juncus* spp.) periodically grazed by sheep and (B) low silt island in the centre of a shallow lake used by roosting waterfowl and covered by bryophytes and Poaceae spp. A randomly placed quadrat (1x1 m) was placed within each habitat type and three replicate subsamples were taken at random from within it. Each sample comprised about 20 g of leaf litter (0 – 2.5 cm) and an equivalent amount from the soil horizon directly below the leaf litter horizon (2.5 – 5 cm). All samples were frozen prior to laboratory analysis.

### 2.2 *Slide preparation and protist counting*

#### 2.2.1 *Diatoms*

A known weight of sample was oven-dried at 105 °C for 48 hours. The range of sample used varied between 0.1 g for leaf litter to 1 g for samples with high mineral content. Diatom samples require digestion to remove organic matter. In this study diatom frustules were treated with 50 ml of 30 % hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to remove organic matter. Hydrochloric acid (10 % HCL) was added to remove calcium carbonates and the supernatant was removed after >12 hours of sedimentation. Samples were re-suspended and cleaned in 30 ml de-ionised water and a 0.3 ml aliquot of the diatom suspension was dried on a glass cover slip and the clean diatom valves were mounted in Naphrax. Diatoms were identified to species level and abundance was measured as the number of individuals per unit dry matter. Diatom frustules were counted under oil immersion at 1000 x magnification using a Meiji Techno microscope. Where possible, a minimum of 400 frustules were counted and identified when more than half the valve was intact. As a result of the differences between aquatic and soil diatoms (Schuttler

1986, Lund 1946), the taxonomy of soil diatoms mainly followed that of Lund (1946). Micrographs illustrating how we defined the various morpho-species can be found in the Appendix of Creevy (2013).

### 2.2.2 *Testate amoebae*

To extract TA a known weight (ranging between 0.1 g for leaf litter to 1 g for samples with high mineral content) of oven-dried (50 °C) sample was soaked and disaggregated in 50 ml de-ionised water and stirred occasionally. The material was washed through a sieve (250 µm) to remove plant and coarse particulate matter. The material was washed into a 50 ml centrifuge tube and centrifuged at 3000 rpm for three minutes and the supernatant was discarded. Samples were stained with Rose Bengal in order to allow the identification of dead and live individuals. TA were identified to species level and abundance was measured as the number of individuals per unit dry matter. Amoeba tests were identified and counted at 200 x and 400 x magnification using a Meiji Techno microscope. Search effort was restricted to 150 individuals per sample (following Davis and Wilkinson, 2004; Payne and Mitchell, 2009; Booth et al., 2010). Morphological identification of TA is based on the guides of Charman et al. (2000), Clarke (2003), Scott et al. (2001), Ogden and Hedley (1980) and Mazei and Tsyganov (2006) as well as some identification keys (Mitchell, unpublished). For some samples SEM (Scanning Electron Microscope) pictures were taken to confirm the identification. For this 0.2 ml of the same solution used for optical microscopy was placed onto aluminium stubs and the samples were air-dried in a desiccator for 3 days prior to SEM analysis. As with the diatoms, described above, micrographs of many taxa – illustrating how the morpho-species were interpreted – can be found in the Appendix of Creevy (2013) available online from Liverpool John Moores University.

### 2.3 *Quantification of protophytic and protozoic Si pools*

Protophytic Si pools represented by diatoms were quantified by multiplying the density of biogenic silica (2.35 g cm<sup>-3</sup>) with corresponding volumes (µm<sup>3</sup>) of silicified frustules (see, e.g., Conley et al., 1989). In order to avoid very time-consuming volume measurements of every single diatom, diatom volumes (µm<sup>3</sup>) and lengths (µm) of a sample of 45 pennate diatoms were measured using a laser scanning microscope (Keyence VK-X110, magnification 200-2.000 x) at ZALF in Müncheberg (Germany). For laser scanning microscopy diatoms were taken from soil suspensions by micromanipulation, washed in distilled water and placed on clean object slides. After air drying images of diatoms were acquired and analysed. Details on laser scanning microscopy can be found in Puppe et al. (2016). Several size categories represented by the length of pennate individuals (smallest size category: 21-25 µm, biggest size category: 96-100 µm) of diatoms were determined and corresponding mean volumes per size category were calculated using an exponential function. Subsequently, this allowed only the length of pennate diatoms to be measured, so a relatively fast scanning of soil suspensions under the light microscope was possible. Due to the fact that diatoms were air dried for analyses under the laser scanning microscope only total diatom but no frustule volumes could be measured because entire frustule dimensions (i.e. frustule thickness and shape) were not visible in most

cases. This is why corresponding silica contents were calculated assuming that only 10 % (conservative estimate, see, e.g., Sicko-Goad et al., 1984) of the complete diatom volume represent the frustule. As biogenic siliceous structures generally consist of hydrated amorphous silica ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ), an average water content of about 10 % was assumed to avoid an overestimation of protophytic Si pools (see Mortlock and Froelich, 1989). Protozoic Si pools represented by euglyphid TA were quantified by multiplication of known silica contents per shell of diverse taxa (Aoki et al., 2007) with corresponding individual numbers (living plus dead TA, for details see Puppe et al., 2014, 2015). Silica ( $M = 60.08 \text{ g mol}^{-1}$ ) contents of diatom frustules and TA shells were converted to Si ( $M = 28.085 \text{ g mol}^{-1}$ ) contents by multiplication with 28/60. In a final step protophytic and protozoic Si pools were converted to dry mass (dm) and given as concentration ( $\text{ng Si g}^{-1} \text{ dm}$ ).

#### 2.4 Numerical analysis

The Shannon's diversity Index (SDI) (Shannon, 1948) was used to examine the microbial diversity of the taxa found in each sample and diversity statistics were obtained using PAST software (Hammer et al., 2001). Formal normality tests in such a context is not recommended (although widely used) and has very low power for small (i.e. <50) sample sizes (Ruxton et al., 2015). Therefore, we have been conservative and mainly used non-parametric Mann–Whitney U test and Kruskal-Wallis test to investigate differences in taxon richness, Shannon's diversity and abundance of TA and diatoms between different habitats and also between leaf litter and soil. Using the General Linear Model (GLM) for One-way Analysis of Variance (ANOVA) we found TA taxon richness and Shannon's index data were normally distributed and met the assumptions for homogeneity of variance, therefore, we used parametric tests including Tukey's post-hoc tests to investigate differences in diversity indices between habitats. Spearman's correlation ( $r_s$ ) was used to test relationships in taxon richness and BSi pools between habitats and through the vertical soil profile (i.e. litter and soil). Statistical analyses were performed using SPSS (version 22.0 IBM Corp.).

### 3. Results

A total of 93 soil protists from two functional groups were identified in leaf litter and soils from a range of habitats across the nature reserve. These comprised of 41 TA (19 Euglyphida and 22 Arcellinida) and 52 diatom taxa. Despite the higher taxon richness in diatoms (many of which were aquatic forms found in bryophytes and soils on an island within a shallow lake), the overall relative abundance of TA individuals was higher (59.3 %) compared with diatoms (40.7 %). With the exception of bryophytes, there was a general trend of higher TA taxon richness in litter and soil substrates compared with diatoms across all terrestrial habitats (Fig. 1a and b). The median testate taxon richness in litter and soil ( $n = 24$ ) was 12.0 with interquartile ranges of 9.5 to 15.0 taxa compared to diatoms ( $n = 24$ ) median taxon richness of 2.0 with interquartile ranges of 0 to 11 taxa. Median Shannon's diversity was also higher for testates (Index = 2.0) with interquartile ranges of 1.8 to 2.2 compared to diatoms (Index = 0.6) with interquartile ranges of 0 to 2.1. There were statistically significant differences found between TA and diatom taxon richness ( $U = 127.5000$ ,  $p < 0.001$ ) and Shannon's diversity ( $U = 144.000$ ,  $p = 0.003$ ). TA were detected in all leaf litter and soil samples in contrast with



diatoms which were not detected in litter and soils associated with acidic, mor humus soils, specifically, those associated with mature *P. sylvestris* woodland.

[Figures 1a and b]

### 3.1 *Community composition*

#### 3.1.1 *Diatoms*

The most abundant taxa were: *Navicula seminulum* (20.5 %), *Hantzschia amphioxys* (7.9 %), *Achnanthes minutissima* (7.0 %) and *Navicula minima* (6.9 %). The median diatom taxon richness was 2.0 with interquartile ranges of 0 to 11.0 with the minimum and maximum number of taxa per sample ranging from an absence of diatom taxa in coniferous woodland to thirty one taxa in litter and soils associated with bryophytes on an island within a shallow lake. Excluding bryophytes, the median taxon richness in deciduous woodland, coniferous woodland and grassland habitats collectively was 1.0 with interquartile ranges of 0 to 4.25. Median Shannon's diversity was 0.6 with interquartile ranges from 0 to 2.1 and ranged between 2.5 (interquartile range 0.5) in bryophytes to 0.28 (interquartile range 0.84) in deciduous woodland. There were statistically significant differences in taxon richness ( $X^2(2) = 19.335, p < 0.001$ ) and Shannon's diversity ( $X^2(2) = 17.434, p < 0.001$ ) between the four habitats studied.

#### 3.1.2 *Testate amoebae*

The most abundant taxa were: *Trinema enchelys* (18.3 %), *Trinema lineare* (13.1 %), *Euglypha rotunda* (12.6 %), *Trinema complanatum* (7.6 %) and *Euglypha tuberculata* type (6.4 %). The median testate taxon richness was 12.0 with interquartile ranges of 9.5 to 15.0 with the minimum and maximum number of taxa per sample ranging from four taxa in grassland soils to seventeen taxa found in soil associated with bryophytes on an island within a shallow lake respectively. Median Shannon's diversity was 2.0 with interquartile ranges of 1.8 to 2.2 and ranged from 2.2 (interquartile range 0.4) in coniferous woodland to 1.8 (interquartile range 0.3) in grassland. ANOVA was used to test for differences in mean taxon richness and Shannon's diversity index between the four habitats studied and there were statistically significant difference in taxon richness ( $F_{3,20} = 6.242, p = 0.004$ ) and Shannon's diversity index ( $F_{3,20} = 3.526, p = 0.034$ ) between the four habitats. Tukey's post hoc tests revealed that grassland diversity indices were significantly different from other habitats studied ( $p < 0.05$ ).

### 3.2 *Protophytic and protozoic Si pools*

Diatom volumes were significantly correlated with diatom lengths ( $r_s = 0.868, p < 0.001$ ) showing a strong exponential relationship. Calculated Si contents of diatom frustules ranged between 127 pg and 1,876 pg for smallest (21-25  $\mu\text{m}$ ) and biggest (96-100  $\mu\text{m}$ ) diatom size categories, respectively (data not shown). Protophytic Si pools (Fig. 2a) were significantly smaller in both litter and soil substrates compared with protozoic Si pools (Fig. 2b) ( $U = 74.000, p < 0.001$ ). Protozoic Si pool was greatest in leaf litter and soils associated with early successional deciduous woodland (relative proportion 34.2 %) compared with other habitats studied. There was no difference in protozoic Si pools between habitats ( $X^2(3) = 6.687, p =$

0.083). In contrast, there was a significant difference in protophytic Si pools between habitats ( $X^2(3) = 19.861, p < 0.001$ ). There was a significant positive correlation between euglyphid taxon richness and protozoic Si pool ( $r_s = 0.668, p < 0.001$ ). The relationship between diatom taxon richness and protophytic Si pool followed a similar pattern with a stronger positive correlation ( $r_s = 0.988, p < 0.001$ ) (Figs. 3a and b). Overall, we found no statistical difference in protist Si pools in the leaf litter compared with the soil horizon. Protophytic Si pool was higher in soils ( $18.0 \pm 7.0 \text{ ng Si g}^{-1} \text{ dm}$ ) compared with the organic leaf litter horizon ( $9.8 \pm 5.5 \text{ ng Si g}^{-1} \text{ dm}$ ), although the high standard errors indicate considerable variability in diatom Si pools in the vertical soil profile. The trend was reversed for testates, we found protozoic Si pool highest in the organic leaf litter horizon ( $63.2 \pm 6.3 \text{ ng Si g}^{-1} \text{ dm}$ ) compared with the soil horizon ( $44.2 \pm 8.7 \text{ ng Si g}^{-1} \text{ dm}$ ) with high variability in BSi pools between samples. In terms of overall BSi pools, the most important diatom taxon was *Hantzschia amphioxys*, accounting for a substantial proportion (46.3 %) of the total protophytic Si pool, while *Trinema enchelys* and *Trinema lineare* were the most important taxa in the protozoic Si pool with proportions of 23.9% and 16.2 %, respectively. The mean relative contributions of different taxa to the overall protophytic and protozoic Si pool in the various habitats is provided (Table 1).

Figures 2a and b

Figures 3a and b

#### 4. Discussion

Grime (1998) emphasised that often it is the taxa with the greatest biomass that drive key ecosystem processes, and the same argument can be made for the whole Earth system (Wilkinson, 2006). Soils harbour a copious abundance and diversity of protists across all known eukaryotic supergroups (Geisen et al., 2015) and better understanding of their distribution in various habitats will lead to a better understanding of their functional roles in different soil systems. In this study we identified over ninety soil protists belonging to two major eukaryotic functional groups, this adds substantial cryptic diversity (sensu Esteban and Finlay, 2010) to the reserves biota. We show the inclusion of just two groups of protists into a biodiversity survey on a single nature reserve has led to a better understanding of the potential of terrestrial diatoms and TA to create Si hotspots in soil. The importance of including soil biodiversity as a criterion in management of conservation land for human health was recently highlighted by Wall et al. (2015) and this is especially relevant to Mere Sands Wood nature reserve which is surrounded by intensive arable agriculture which likely has an impoverished soil microbial community. Recent studies (for example, Lara et al., 2015; Geisen et al., 2015) have shown that the use of modern molecular approaches can be extremely powerful and effective methods to recognise and quantify soil protists and can be used in conjunction with morphology-based taxonomy approaches. Our preliminary results are in agreement with comparative molecular data (Geisen et al., 2015) suggesting Amoebozoa are perhaps more important in grassland and forest soils compared with Diatoms (Stramenopiles). The combination of an inter-disciplinary approach along with the inclusion of microorganisms and microscopic metazoan fauna into biodiversity surveys will almost certainly lead to a better, more comprehensive understanding of microbially driven ecosystem processes. In interpreting

our data it should be remembered that this is a snapshot of protist diversity at just one time in the year (September) and that protist numbers vary throughout the year (Couteaux, 1976; Mitchell et al., 2008).

Very few studies have quantified both TA and diatoms collectively in the soil environment. To our knowledge there is only one study which assessed the usefulness of both groups for biomonitoring in agricultural soils and found them sensitive to different farming practices (Heger et al., 2012). Considering that diatoms and some testates both utilize Si for shell construction, we might expect the diversity and abundance of one group to follow that of the other but the results of this study do not support this idea. Overall, we found that TA were more abundant in leaf litter and soils compared with diatoms. TA were identified in all habitats, whereas diatoms were not identified in leaf litter and soils associated with mature coniferous woodland. This is perhaps not surprising as light levels are low on this woodland floor and light is usually considered a prerequisite for diatom production, although mixotrophy has occasionally been described in freshwater ecosystems (Bavestrello et al., 2000). It is recognised that some species of TA harbour photosynthetic symbionts and these mixotrophic taxa are especially important in nutrient-poor environments (Gomaa et al., 2014), but there are no records of photosynthetic endosymbionts in euglyphid TA, suggesting that light may not be a factor for these testates. The results are in agreement with Wilkinson and Mitchell's (2010) suggestion that TA have a particularly important role in nutrient cycling in mor humus type soils.

Results from microcosm and field studies indicate that microbial species diversity, functional group diversity and community composition can all influence ecosystem process rates (McGuire and Treseder, 2010). In the present study we found strong relationships between protist taxon richness and soil BSi pools. This result indicates that protist diversity and community composition can influence terrestrial BSi pools and provides a better understanding of the eukaryotic microbial mechanisms involved in Si cycling in terrestrial environments (Opalinka and Cowling, 2015; Alfredsson et al., 2015). The species richness-productivity relationship (SRPR) has been extensively studied in plants (Šímová et al., 2013), and to a lesser extent in prokaryotes (Zhalnina et al., 2015), but far fewer studies consider the eukaryotic SRPR relationship. In terms of run-off of terrestrial Si into waterbodies, Wilkinson and Mitchell (2010) suggest that testates with siliceous idiosomes (such as *Euglypha* spp.) could potentially have increased the rate of Si mineralization in soils with direct implications for nutrient availability for diatoms in aquatic ecosystems. To better understand future effects of human-induced land-use change on ecosystem processes, such as the Si cycle (see, e.g., Vandevenne et al., 2015), we need to better understand the microbes involved and where Si is accumulated in the landscape (Sommer et al., 2006). As (alkaline) extraction methods (an overview is given in Sauer et al. 2006) are hardly able to differentiate between various BSi pools, direct (microscopical) methods are needed for a more accurate quantification of single (e.g. phytogenic, protophytic, protozoic) BSi pools. Furthermore, we show that Si-contents are species-related (or at least size-related), so it is necessary to get information on population composition. Here we demonstrate that population size data provides a mechanism for understanding protist functional roles in natural soils. Future research should focus on the

mechanisms by which microbial diversity influences Si cycling in terrestrial environments, in particular, the rate of Si turnover in testates and diatoms to determine the relative importance of protists to the Si pool.

This morphology-based approach which characterises protozoic and protophytic population sizes and calculates corresponding BSi pools provides one example of how we can differentiate between different BSi pools in soil systems (Sommer et al., 2006). However, we do acknowledge that our results provide an indirect measure of protozoic and protophytic soil Si pools and do not take into account accumulation, redistribution and losses and gains of Si during soil development. In this study we compared BSi pools represented by euglyphid TA and diatoms and recent molecular work (Lahr et al., 2015) suggests the protist Si pools calculated here could be underestimated as some arcellid TA also produce Si rich shells. Interestingly, we found a similar trend in BSi pools and taxon richness across different habitats. In particular, higher taxon richness in testates and diatoms were observed in early successional vegetation (i.e. bryophytes and early successional deciduous woodland) and this corresponded to greater BSi pools, potentially creating more Si hot spots in these soils. The results of Puppe et al. (2014) showed that the protozoic Si pool is built up rapidly at very young (< 10 yrs) initial ecosystem states and is strongly linked to plant growth. In soils of forested ecosystems protozoic Si pools were larger compared to the ones in initial ecosystems and could reach up to 5 kg Si ha<sup>-1</sup> (Puppe et al., 2015). Annual biosilicification rates of living TA (17–80 kg Si ha<sup>-1</sup>) were comparable to or even exceeded reported data of annual Si uptake by trees (Puppe et al., 2015; Sommer et al., 2013). As this study was originally conceived to estimate taxon diversity (rather than focusing on the Si cycle) we did not determine bulk densities and so are unable to calculate BSi pools per hectare or square meter - for example, such data can be found in Puppe et al. (2014, 2015) or Sommer et al. (2013).

Compared with protozoic Si pools, fewer studies consider the role of protophytic Si pools in terrestrial environments, although the potential of diatoms for Si cycling, especially in wetlands, has been recognised (Clarke, 2003; Kokfelt et al., 2009; Struyf and Conley, 2009; Struyf et al., 2010). Aoki et al. (2007) demonstrated the potential importance of amoebal Si pools to Si cycling in terrestrial environments because of their small-size, rapid turnover time and sheer abundance in soil. In this study, the larger soil diatoms, specifically *Hantzschia amphioxys* tended to have the greatest proportion of the protophytic Si pools. In contrast, the highest proportion of BSi pools in soil testates were found amongst the small, bacterivorous, pioneer taxa, for instance, *Trinema enchelys* and *Trinema lineare*, which collectively made up a significant proportion (40.1 %) of the total protozoic Si pool. Diatoms take up dissolved Si, convert it to biogenic forms for frustule formation, with Si being mobilized again upon decomposition (Opalinska and Cowling, 2015). Therefore, as with TA, they are increasing Si mineralisation and hence, the biological availability of this element for subsequent plant uptake (Wilkinson, 2008).

Overall, we found no statistical difference in protist Si pools in the leaf litter compared with the soil horizon. Alfredsson et al. (2015) found that soil diatoms were important components of soil amorphous silica storage in the top organic horizon of peatlands and less so in the

mineral horizons of shrub tundra. In this study we found diatom Si pools were higher in soils compared with the organic leaf litter horizon which may be due to several compounding and auto correlating factors, for example, 1) less light in the pine dominated organic, acidic soils, 2) greater year round interstitial moisture content and 3) greater nutrient availability in the mineral soils, thus promoting greater diatom productivity and hence Si uptake. The high standard errors indicate considerable variability in diatom Si pools in the vertical soil profile. The trend was reversed for TA, where we found euglyphid Si pools highest in the organic leaf litter horizon. For grassland, low standard errors suggest that these taxa are distributed in a relatively uniform manner through these soils. In contrast, for coniferous and deciduous woodlands large standard errors indicate a more patchy distribution and suggest that further research is needed to explain the variability in protist Si pools. Opalinska and Cowling (2015) also found large variations in BSi pools in coniferous catchments suggesting pH is a very important factor in altering BSi dissolution, for instance, once you start to get alkaline soils Si becomes much more soluble and available. We hypothesize that hot spots of Si from decaying TA shells etc. may be more valuable to plants in acidic soils when Si is less available from parent material. Importantly, if we are to improve the development of Si cycling models (Opalinska and Cowling, 2015), it is essential that we quantify protist Si pools and incorporate this data into existing models because of the potential for these microorganisms to create Si hot spots in the soil.

## **5. Conclusion**

We conclude that quantification of microbial diversity in soils is vital in order to begin to estimate the full diversity of life on a nature reserve or other location. However, species lists are not enough – in order to start to understand the functioning of microbes in the Earth system we need to quantify population sizes. Applying such data to questions on soil BSi we found that protophytic and protozoic Si pools might be significant for creating BSi pools/hot spots, particularly in initial ecosystem states and are linked especially to vegetation. Further research into the eukaryotic SRPR is needed to better understand links between microbial diversity and ecosystem function. As our provisional calculations for BSi pools illustrate, once population data on microbial taxa are available it starts to be possible to attempt to quantify their role in various ecological processes such as the Si cycle.

## **Acknowledgements**

We wish to thank Lindsay Beaton, Reserve Manager at Mere Sands Wood Nature Reserve and the Wildlife Trust for Lancashire, Manchester and North Merseyside for allowing us permission to carry out this study. D.P. was partly funded by the German Science Foundation (Deutsche Forschungsgemeinschaft, DFG) project ‘Spatiotemporal dynamics of biogenic Si pools in initial soils and their relevance for desilication’ (SO 302/7-1). We would like to thank Stefan Geisen and two anonymous reviewers for their helpful comments on our manuscript.

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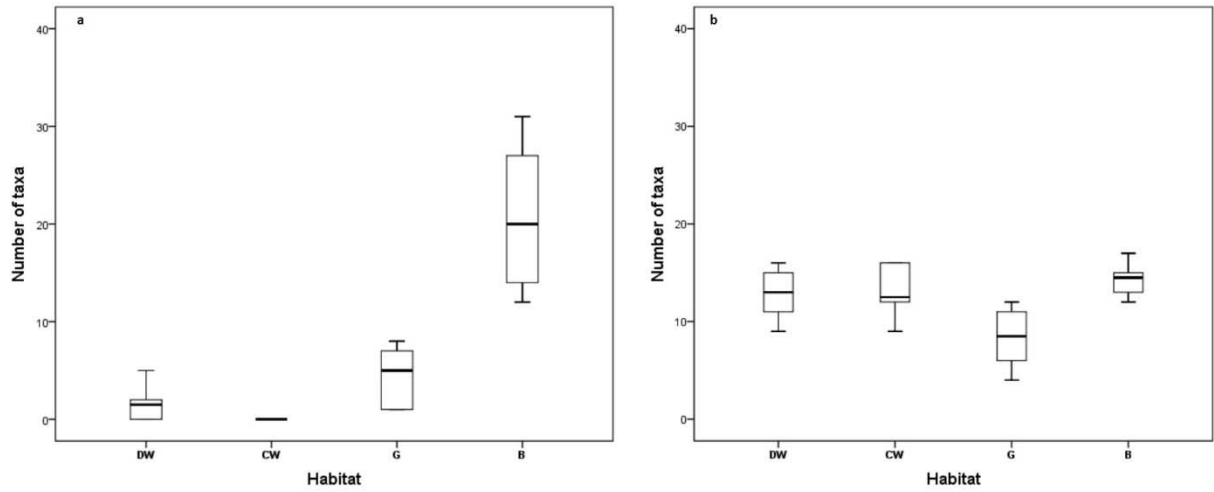
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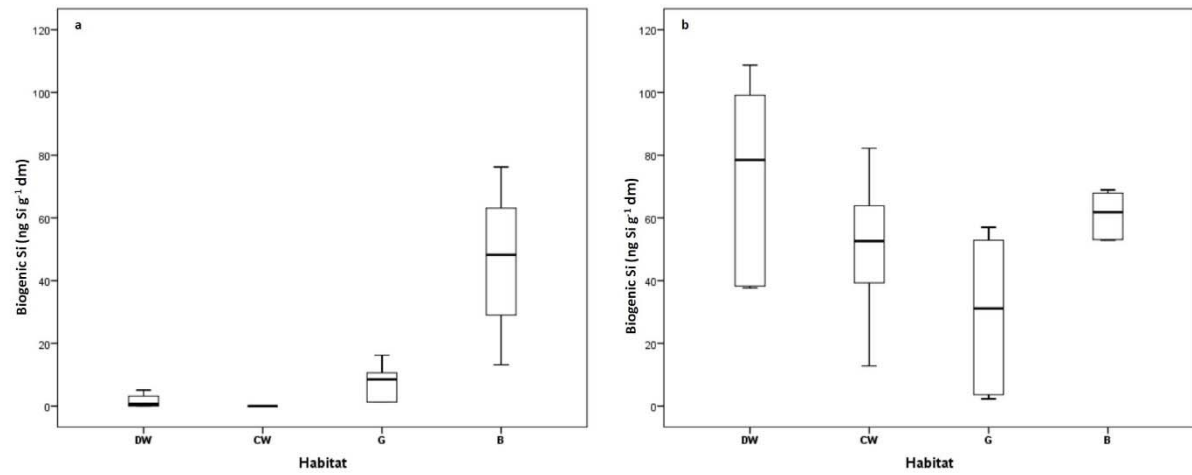
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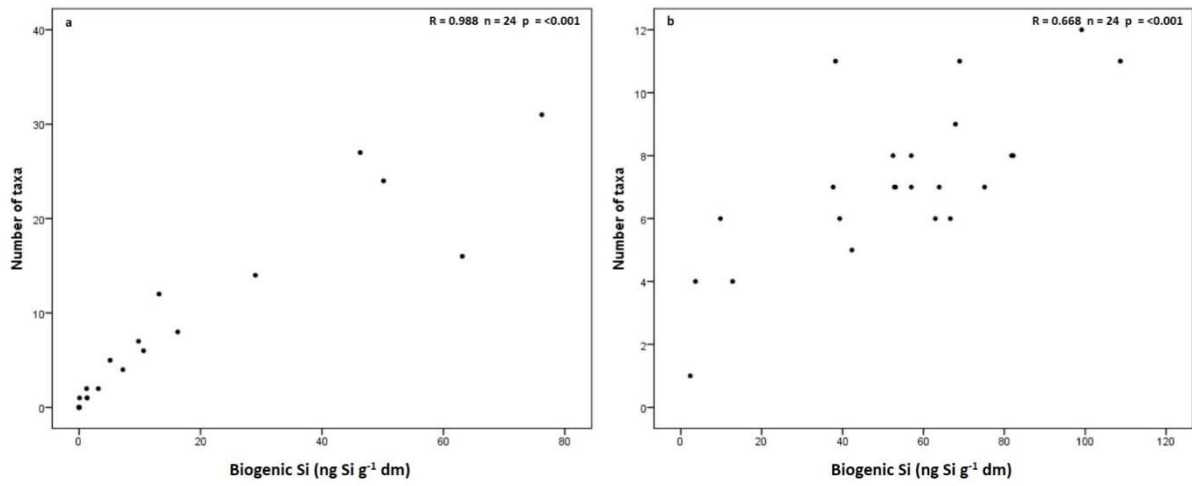
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Figures 1a and b. Boxplots showing differences in diatom (a) and testate amoebae (b) taxon richness between four habitats on a nature reserve. The top, mid and bottom line represent the 75<sup>th</sup>, 50<sup>th</sup> and 25<sup>th</sup> percentiles and the horizontal lines represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles.



Figures 2a and b. Boxplots showing differences in diatom (a) and testate amoebae (b) silica pools between four habitats on a nature reserve. The top, mid and bottom line represent the 75<sup>th</sup>, 50<sup>th</sup> and 25<sup>th</sup> percentiles and the horizontal lines represent the 10<sup>th</sup> and 90<sup>th</sup> percentiles.



Figures 3 a and b. Relationship between microbial taxon richness and Si pools (ng Si g<sup>-1</sup> dm) in a) diatoms and b) testate amoebae

**Table 1a.** Soil pH and bulk organic matter (OM) content with protophytic and protozoic diversity statistics and corresponding BSi pools (ng Si g<sup>-1</sup> dm) in leaf litter (0 - 2.5 cm) and soils (2.5 - 5 cm) at Mere Sands Wood nature reserve (DW=deciduous woodland, CW=coniferous woodland, G=poaceae, B=bryopytes). Values are means (n=3) with standard errors in brackets.

	DW		CW		G		B	
	0 - 2.5	2.5 - 5	0 - 2.5	2.5 - 5	0 - 2.5	2.5 - 5	0 - 2.5	2.5 - 5
Bulk OM (wt. %)	48.4 (9.4)	4.4 (0.0)	95.3 (1.3)	95.6 (0.3)	51.1 (7.8)	17.5 (1.9)	81.7 (8.4)	71.9 (4.5)
pH (CaCl <sub>2</sub> )		6.1 (0.5)		4.1 (0.3)		6.4 (0.1)		-
Protophytic taxon richness	0.3 (0.6)	3.0 (1.7)	0	0	3.0 (3.5)	6.0 (2.0)	14.0 (2.0)	27.3 (3.5)
Protophytic Shannon index	0	0.9 (0.5)	0	0	0.6 (1.0)	1.6 (0.3)	2.3 (0.2)	2.7 (0.3)
Protophytic Si pool (ng Si g <sup>-1</sup> dm)	0.2 (0.2)	3.2 (1.1)	0	0	4.1 (2.8)	11.3 (2.6)	35.1 (14.7)	57.6 (9.4)
Protozoic taxon richness	11.3 (0.6)	7.3 (0.6)	6.3 (1.5)	5.7 (1.5)	7.3 (0.6)	3.7 (2.5)	7.3 (0.6)	8.7 (2.5)
Protozoic Shannon index	2.2 (0.3)	1.8 (0.3)	2.1 (0.2)	2.2 (0.3)	1.9 (0.1)	1.6 (0.2)	2.1 (0.1)	2.2 (0.2)
Protozoic Si pool (ng Si g <sup>-1</sup> dm)	82.0 (22.1)	64.9 (13.7)	62.5 (11.5)	38.7 (14.7)	54.1 (1.4)	5.2 (2.3)	54.3 (1.3)	67.8 (0.7)

**Table 1b.** Mean relative proportion (%) with standard errors (in brackets) of diatom taxa contributing to the protophytic Si pool (ng Si g<sup>-1</sup> dm) in litter (0 - 2.5 cm) and soil (2.5 - 5 cm) at Mere Sands Wood nature reserve (DW=deciduous woodland, CW=coniferous woodland, G=poaceae, B=bryopytes). Values are means (n=3).

	DW	CW	G	B
<i>Achnanthes thermalis</i>	0	0	0	0
<i>Achnanthes minutissima</i>	0	0	0	0
<i>Cocconeis placentula</i>	0	0	0	0

<i>Hantzschia amphioxys</i>		0	0		0	0		25.9 (14.8)	19.7 (3.7)		16.1 (10.1)	19.2 (6.5)
<i>Hantzschia virgata</i>		0	4.1 (4.1)		0	0		0	0		3.8 (2.2)	1.8 (0.3)
<i>Navicula cinca</i>		0	0		0	0		0	0		0.7 (0.7)	0.9 (0.5)
<i>Navicula minima</i>		0	0		0	0		0.4 (0.4)	0.3 (0.3)		0.3 (0.2)	0.9 (0.3)
<i>Navicula seminulum</i>		0	0		0	0		0	0		1.0 (0.4)	3.0 (0.5)
<i>Nitzschia inconspicua</i>		33.3 (26.5)	0		0	0		0	0		2.5 (1.5)	0.9 (0.2)
<i>Pinnularia borealis</i>		0	29.3 (8.4)		0	0		0	0		0.3 (0.3)	0
<i>Pinnularia microstauron</i>		0	0		0	0		1.8 (1.8)	4.5 (2.4)		0.07 (0.07)	0
<i>Pinnularia viridis</i>		0	0		0	0		5.2 (5.2)	8.9 (3.4)		5.0 (2.6)	2.6 (0.8)
<i>Stauroneis kriegerii</i>		0	0		0	0		0	0		0.3 (0.3)	1.0 (0.2)

**Table 1c.** Mean relative proportion (%) with standard errors (in brackets) of testate amoebae taxa contributing to the protozoic Si pool (ng Si g<sup>-1</sup> dm) in litter (0 - 2.5 cm) and soil (2.5 - 5 cm) at Mere Sands Wood nature reserve (DW=deciduous woodland, CW=coniferous woodland, G=poaceae, B=bryopytes). Values are means (n=3).

<i>Assulina muscorum</i>		0.4 (0.3)	0		0.7 (0.7)	0.4 (0.4)		0	0		0	0
<i>Assulina seminulum</i>		2.4 (1.2)	0.1 (0.1)		2.5 (0.8)	4.8 (2.5)		0	0		0	0
<i>Corythion dubium</i>		10.2 (5.0)	0.3 (0.3)		0.7 (0.6)	1.3 (1.3)		0	0		0	0.4 (0.4)
<i>Euglypha cristata</i>		0.2 (0.2)	0.1 (0.1)		0	0		0	0		0	1.9 (1.2)
<i>Euglypha laevis</i>		0.3 (0.1)	0.5 (0.5)		0	0		0.6 (0.2)	0		1.4 (0.1)	1.7 (0.6)
<i>Euglypha rotunda</i>		1.3 (0.2)	2.3 (0.4)		3.9 (1.2)	2.3 (1.6)		2.8 (0.7)	4.9 (3.5)		7.0 (1.0)	8.0 (3.1)
<i>Euglypha strigosa</i>		8.6 (4.4)	0		4.0 (0.7)	0		0	0		0	0
<i>Euglypha tuberculata</i> type		2.7 (0.5)	3.5 (2.2)		0	0.4 (0.4)		5.5 (2.6)	0		5.2 (2.5)	6.5 (1.4)
<i>Trinema complanatum</i>		0.4 (0.2)	9.9 (5.5)		4.4 (0.6)	5.8 (1.1)		0	0		0	0
<i>Tracheleuglypha dentata</i>		0.1 (0.1)	0.9 (0.5)		0	0		4.2 (0.3)	5.3 (5.3)		0.8 (0.8)	0.9 (0.7)
<i>Trachelocorythion pulchellum</i>		1.8 (0.2)	0.4 (0.4)		0.2 (0.2)	3.4 (2.2)		1.0 (0.1)	1.4 (1.4)		1.5 (0.2)	2.7 (0.5)
<i>Trinema enchelys</i>		3.3 (1.3)	9.5 (2.8)		16.8 (6.5)	14.9 (7.1)		14.3 (1.9)	12.6 (1.8)		10.0 (1.3)	6.1 (1.6)

<i>Trinema lineare</i>		1.7 (0.4)	5.8 (2.1)		0	0		5.0 (0.5)	9.2 (5.9)		7.5 (0.8)	5.0 (0.8)
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